THE SEGREGATION AND RECOMBINATION OF HOMOLOGOUS CHROMOSOMES AS FOUND IN TWO GENERA OF ACRIDIDAE (ORTHOPTERA)

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FIVE TEXT FIGURES AND FOURTEEN PLATES

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[1] Contribution from the Zoological Laboratory of the University of Pennsylvania.

I. INTRODUCTION

1. Statement of the problem

The present trend towards a closer correlation of the subjects of cytology and genetics is one that promises much mutual benefit, as is well demonstrated by the work of T. H. Morgan and his co-laborers at Columbia University. While the present work deals entirely with cytological data, the material promises much for a genetical study which, it is hoped, may be undertaken later.

Early in the course of his studies on Acrididian spermatogenesis Dr. McClung outlined a general plan of study which, it is hoped, will lead to a correlation between chromosomes and body characters. This plan involves (1) a study of the chromosomes of selected species of closely related genera; (2) a study of numerous species within a genus; and (3) a study of the details of organization of the chromosomes of certain species.

In pursuance of this plan McClung has worked on the genus Hesperotettix; Nowlin, taking up the genus Melanoplus belonging to the same group, has published ('08, '14) her results on five species of this genus; Robertson ('16) has carried on work on the Tettiginae; Pinney ('08) and Wenrich ('16) have studied the genus Phrynotettix; and the writer has begun work on the genus Trimerotropis—subfamily Oedipodinae.

It is my purpose to confine this paper as closely as possible to its own special topic. Two hypotheses, put forward by Van Beneden ('83) and elaborated by Boveri ('88, '02) are, however, essential to an interpretation of the facts presented. The first, that of the individuality of the chromosomes, also supported by Rabl ('85), has been actually demonstrated for the individual.

at least, in one of the short-horned grasshoppers, Phrynotettix magnus, by Wenrich ('16) who, through years of close study, has been able to follow one well marked individual chromosome pair from the spermatogonia through the most diffuse stages to the spermatids. The other one-time hypothesis of Van Beneden ('83) that one-half of the chromosomes are of paternal and one-half of maternal origin was clearly shown to be a fact by the late Carl Mulsow ('12) in his study of the gametogenesis and fertilization of a parasitic trematode, Ancyracanthus, in which the chromosomes may be counted, even in the living spermatozoon as it enters the egg and fuses with the female pronucleus.

Sutton ('02) was the first to furnish actual support for the latter hypothesis and to suggest a thorough-going correlation of the chromosomes and somatic characters; he recognized in Brachystola magna a double series of chromosomes in the spermatogonia which he considered to be of biparental origin. He indicated the probable relationship between Mendelian phenomena and the possible distribution of the chromosomes in the gametes and their recombination in the zygote. But, so long as the homologous chromosomes of this double series were indistinguishable, it was impossible to follow, cytologically, the chromosomes derived from a given parent and to determine their manner of segregation in relation to any other chromosomes.

As is well known, in the Orthoptera and many other animals as well, the first maturation division determines which of the derivative cells will, in fertilization, produce a male and which a female, since the accessory chromosome—the sex determinant—passes undivided into one of the daughter cells at this time. While probably it is purely a matter of chance which of the daughter cells the accessory enters—that is, it parallels any Mendelian character in the matter of segregation—nevertheless, it marks unalterably, after it has passed to one pole, the male from the female producing spermatozoon. If one of the homologues of any pair is recognizably different from its mate and these homologues should segregate from each other at the first maturation division their manner of segregation in relation to sex would be apparent. The only instances of this sort to be

reported, so far, are those in which the homologues differ markedly in size. The first was the work of the writer which appeared in 1913, giving data showing the alternative distribution of the unequal dyads of one tetrad in relation to sex in certain Oedipodinae. Three hundred first spermatocytes were counted; in 51.3 per cent the larger dyad was going into the same second spermatocyte as the accessory, and in 48.7 per cent the smaller dyad was accompanying the accessory. Shortly afterward a paper by Voïnov ('14) appeared, giving like results and an essentially similar conclusion from a study of Gryllotalpa vulgaris.

Wenrich, in 1914, reported similar results from a count of four hundred and seventy-two first spermatocytes of Phrynotettix magnus where a tetrad with unequal dyads is also present. And lastly, Robertson ('15), in an addendum to a paper in which he reports an unequal pair of chromosomes in Tettigidea parvipennis and in Acridium granulatum, states that these unequal pairs agree with the one in my material in regard to the distribution of their dyads in relation to the accessory. These I believe are the only instances so far reported where the chromosomes derived from different parents could be followed in the germ cells and their distribution determined.

It is to this problem of tracing the segregation of certain well marked homologues in given individuals of the species studied and then of determining the combinations actually present in a considerable number of individuals of these species that I wish to devote this paper. My study has been confined to the metaphases, but a knowledge of the behavior of the dissimilar homologues during synapsis is essential to my conclusions. As this problem was aside from my general plan, and directly in line with Dr. Wenrich's work, he kindly undertook its solution. In a paper now in press he shows that the process of synapsis and tetrad formation is the same for the heteromorphic tetrads as for the homomorphic ones, whether the latter have terminal or nonterminal fiber attachment.

2. Nomenclature

The unusual conditions of the chromosomes in this group have made advisable the introduction of four new terms.

1. Homomorphic—used to designate those tetrads made up of morphologically similar homologues.
2. Heteromorphic—used to designate those tetrads made up of morphologically different homologues.
3. Telomitic—a term used to indicate terminal fiber attachment.
4. Atelomitic—a term used to indicate nonterminal fiber attachment.

The two latter are extensions of the ideas involved in the terms 'Hippiscus type' and 'Stenobothrus type' as used by McClung ('14). This has seemed desirable since the work of Robertson ('16) and some recent work of McClung indicates that the chromosomes of Stenobothrus may be fundamentally different from those of the typical Acrididian complex. I shall also apply the terms telomitic and atelomitic to spermatogonial and somatic chromosomes as well as to those of the first spermatocyte. The term Hippiscus type will be used interchangeably with telomitic for those first spermatocyte chromosomes which are comparable in structure to the Hippiscus rings. However, in this material the tetrads of the Hippiscus type are usually transformed into rods in the metaphase as described by McClung ('14). In the same way Stenobothrus type and atelomitic may be used interchangeably.

3. Special fitness of material

The genus Trimerotropis is confined to the American continent and contains over thirty species, the extreme members of which merge with those of four other genera. The present paper deals with the fallax group of the genus and its relation to the genus Circotettix, with especial reference to the heteromorphic tetrads found in each. In regard to the systematic relationships, it is believed that a definite cytological criterion—

difference in number of chromosomes—has been found for separating the two genera.

On this evidence the species suffusus belongs to the genus Trimerotropis instead of to Circotettix, while the heteromorphic tetrads found in both genera may point to a common origin. Furthermore, the evidence, as will be shown, indicates that the fallax group constitutes a single species.

The group which forms the basis of this work is known to taxonomists as one of the most difficult to classify. Certain species of the group have been removed to the genus Circotettix by some taxonomists, while others have split up the remainder into four species. If one assume that the chromosomes are direct descendants from ancestors morphologically like themselves, are the bearers of the determiners of the hereditary characters, and that the homologues actually express, in their architecture, differences in the determiners, the results of a study of the maturation divisions in the male leave little surprise that taxonomists are bewildered.

Dr. McClung has shown ('14) that the point of spindle fiber attachment is normally constant, but at some period in the history of this species there must have been a reorganization to the extent of a shifting of the attachment in certain chromosomes. The most striking result has been to produce J-shaped tetrads in the first spermatocyte. Such shapes are due to one homologue being rod-shaped; that is, it has terminal fiber attachment, while its mate has nonterminal fiber attachment. Of the twelve first spermatocyte chromosomes, ten tetrads and the accessory are affected by this reorganization. If the female is similarly involved and if there is free fertilization, bringing about chance recombination, one might expect to find in a given individual all ten of the tetrads represented by two rod-shaped homologues, giving rod-shaped tetrads, or one might find all of the homologues with nonterminal fiber attachment, resulting in tetrads of the Stenobothrus type (McClung '14). Between these two extremes there might be present every possible combination in J's, rod's and Stenobothrus-like rings.

These expectations have been realized in part; and, furthermore, in two of these ten tetrads there is a third type with both homologues having nonterminal fiber attachment, but with a secondary shifting so that one of the free arms is longer than the other. One finds, besides such heteromorphic tetrads (plate 14, fig. 22*c*), homomorphic tetrads of the two expected types, one with both arms long (plate 14, fig. 23*a*) and the other with both arms short (plate 14, fig. 10*a*).

Another peculiarity, that of a single constriction occurring near the proximal ends (that is, the ends directed towards the poles), may mark certain tetrads. An example may be seen on plate 14, figure 21*a*. One dyad of such a tetrad (Chromosome number 3, plate 4) may be of three types; V-shaped (plate 5, fig. 31), plain rod (plate 5, fig. 32) or constricted rod (plate 5, figs. 31 and 32); these occur in various combinations, furnishing a visible mechanism for possible triple allelomorphs.

4. Methods and acknowledgments

The material for the somatic mitoses of the female was fixed in a modification of Bouin's solution which has been developed in this laboratory and which promises to be very useful. The following is the formula:

Picric acid, saturate aqueous solution	75 cc.
Formalin (full strength)	15 cc.
Glacial acetic acid	10 cc.
Urea, crystals	½ gm.

After immersion in this fluid, material may be removed any time between twenty-four hours and three months. It should then be washed in water some fifteen minutes and run up gradually to 75 per cent alcohol where it may be kept indefinitely. The fact that specimens may be left for a long time in this fixative makes it especially desirable for field work and for those not equipped to make use of more elaborate methods. Acrididian metaphase chromosomes are as well preserved as in Flemming.

The testes were fixed in strong Flemming solution. Sections were cut from 7 to 12 microns in thickness; 10 proved best suited to this work. The stains used were Flemming's tricolor and Heidenhain's iron-hematoxylin.

I wish to thank Dr. McClung for many helpful suggestions and much kindly interest as well as for material from his own collection. It is also through his efforts that I am indebted to the University of Pennsylvania for a collecting trip during the summer of 1915 to obtain additional material. I am, besides, indebted to the Marine Biological Station at Woods Hole for laboratory facilities during the summer of 1914. I also wish to express my thanks to Mr. J. A. G. Rehn and Mr. Morgan Hebard of the Academy of Natural Science for the identification of the specimens and helpful suggestions concerning the taxonomy of the group, and finally to Dr. D. H. Wenrich for much kindly cooperation.

II. OBSERVATIONS

Since the evidence for segregation and recombination must be derived largely from the maturation divisions, I shall begin with the first spermatocytes, then go back to the spermatogonia and to the somatic complexes of the female for corroborative evidence, and finally, take up the second spermatocytes, which are morphologically related directly to the spermatogonial and somatic complexes. The individuals are numbered consecutively. Where more than one complex is shown from an individual the succeeding ones are indicated by letters; e.g., number 1 always refers to a given animal; the letters after the 1 (*a to x*) designate drawings from this specimen.

1. First spermatocytes

a. Segregation of homologues of J-shaped tetrads in individual no. 1, Trimerotropis (?) suffusa (?). This is one of two individuals belonging to what I designate as form 'B.' They were collected on Orcas Island in Puget Sound by a party from Kansas University in the summer of 1909. Both individuals are alike.

Of the twelve first spermatocyte chromosomes (plate 1), four (nos. 9 to 12) are atelomitic—Stenobothrus type (McClung, '14)—four (nos. 2, 3, 5, and 6) are telomitic—Hippiscus type, the accessory (no. 4) is V-shaped, atelomitic, while the remaining three tetrads (nos. 1, 7 and 8) have one dyad of the Stenobothrus type and the other of the Hippiscus type.

Several important questions at once present themselves. 1) Since these heteromorphic but homologous dyads segregate in the first maturation division, what is their distribution in relation to the accessory (that is, to sex)? 2) As it is obvious that at least sixteen sorts of spermatozoa are formed in these two individuals, would the well known constancy of the complex—as shown by numerous workers on Orthoptera—hold for this species or would a large number of individuals give all possible combinations? Or would certain combinations result and others fail? 3) Would the somatic complex of the female be constant? (Using this as an index of the oogonial complex). 4) If the complex is constant for the species, what is the mechanism by which it is regulated? Is there selective fertilization of a most complex sort, or is there free fertilization with regulation occurring at the time of the maturation of the egg? (Since copulation occurs some twenty-four hours before the polar bodies are formed the latter seemed quite possible.)

The first of these questions was a matter to be determined by a study of a given individual and was at once worked out from the material in hand. The others required a considerable number of individuals and it was largely for the purpose of obtaining these that a collecting trip through the southern and western states was undertaken during the summer of 1915.

For a determination of the method of segregation of the heteromorphic homologues in relation to the accessory, one hundred camera lucida drawings of entire complexes were made at random. As stated above, the two individuals in the collection were alike; fifty-seven of the drawings were from one individual and forty-three from the other. Plate 1 is based on only one individual, however, but is representative of both.

These drawings are from sections. The chromosomes from one cell are always in two and sometimes three sections. The sections were all present, in order and in straight rows, so that the problem of identifying in successive sections these large clear cells with sharply formed spindles is much simpler than it may appear to those accustomed to less favorable material. The chromosomes were first outlined under the camera lucida

in their actual relation to each other in the sections, then a careful study was made and details filled in when necessary. Later, the chromosomes represented in plate 1 were arranged roughly according to size and placed on the plate so that the transverse rows represent the chromosomes found in one cell while the vertical rows represent the corresponding chromosomes in different cells. The eight complexes shown on this plate are typical of the conditions found in the hundred cells. They are so placed that the accessory is always passing to the upper pole.

Taking up first the small one at the right (no. 1), which is one of the heteromorphic pairs, we find in five of the cells (*b*, *c*, *d*, *f*, *h*) the atelomitic dyad going to the cell which lacks the accessory, while in three (*e*, *g*, *i*) it is going into the same cell as the accessory. The other two heteromorphic chromosomes, numbers 7 and 8, respectively, are so nearly identical in size and behavior that no attempt was made to distinguish between them. Instead, the segregation of their homologues in relation to each other and in relation to the accessory was noted and gave all of the information desired. If these two chromosomes are compared with each other in the first four cells (*b*, *c*, *d*, *e*), it will be observed that the dyads with nonterminal fiber attachment are going to opposite poles; in the remaining four cells (*f*, *g*, *h*, *i*) they are going to the same pole. But in cells *f* and *g* they approach the pole which lacks the accessory, while in the last two they will enter the same cell as the accessory. Now, if we compare chromosome number 1 with numbers 7 and 8, it will be seen that its dyads also segregate independently of either of the others. For instance, in the last four cells (*f*, *g*, *h*, *i*) its atelomitic dyad passes, either to the same second spermatocyte as the similar dyads of the larger chromosomes (*f* and *i*), or to the cell which receives the telomitic dyads (*g* and *h*). It is evident then that here are four chromosomes (nos. 1, 4, 7, 8) for which this is the segregation division and that they are distributed more or less without regard to each other or to the second spermatocytes.

If there is free segregation, the number of equally possible combinations in the gametes of a single individual is repre-

sented by the formula 2^n in which n represents the number of chromosomes in the reduced series; that is, the number of pairs of homologues. In this instance $n = 4$ since we are considering only the accessory and the three heteromorphic tetrads. Then 2^4, or 16, is the possible number of combinations of these chromosomes in the gametes of this individual. While the number of morphologically different gametes formed as a result of the segregation of any *given* three is 8 (2^3), of any given two is 4 (2^2) of a given one 2 (2^1). The occurrence of *any* combination is shown by the coefficients of the expanded binomial raised to the nth power in which case n again represents the number of homologous pairs. In this instance the series of coefficients is 1–4–6–4–1.

From the two formulae given above we should expect to find

Any *given* 4 V's in $\frac{1}{16}$ of the gametes	*Any* 4 V's in $\frac{1}{16}$ of the gametes
Any *given* 3 V's in $\frac{1}{8}$ of the gametes	*Any* 3 V's in $\frac{4}{16}$ of the gametes
Any *given* 2 V's in $\frac{1}{4}$ of the gametes	*Any* 2 V's in $\frac{6}{16}$ of the gametes
Any *given* 1 V in $\frac{1}{2}$ of the gametes	*Any* 1 V in $\frac{4}{16}$ of the gametes
and 0 V in $\frac{1}{16}$ of the gametes	and 0 V in $\frac{1}{16}$ of the gametes

The difference—it will be noted—between these two series is in regard to *any* one, two or three as opposed to a *given* one, two or three; that is, in the latter case, we must distinguish between the V's with which we are dealing. A given V (as the accessory) would be found in one-half of the gametes, but, on the other hand, one-quarter of the gametes would contain only one V.

Perhaps both may be better shown graphically. Let A, B, C, D, represent the V-shaped homologues and the accessory and a, b, c, d, the rod-shaped homologues of the tetrads and the absence of any homologue in the case of the accessory. There are then the foregoing sixteen combinations any one of which is equally probable; *all* four V's together, e.g., A B C D, one-sixteenth of the time; *any* three, as in the second division, four-sixteenths of the time; any *given* three, e.g., A, B, C, two-sixteenths of the time; *any* two, as in the third division, six-sixteenths of the time; any *given* two, four-sixteenths of the time; while any *given* one occurs eight-sixteenths of the time and *only* one four-sixteenths of the time.

A B C D	a B C D	a b C D	a b c D	a b c d
	A b C D	A b c D	a b C d	
	A B c D	A B c d	a B c d	
	A B C d	a B c D	A b c d	
		a B C d		
		A b C d		

To be specific if A = accessory, B the V-shaped dyad of number 1 and C and D the indistinguishable ones of numbers 7 and 8, then, out of sixteen second spermatocytes, we should have the accessory in eight-sixteenths, the accessory and V of tetrad number 1 in four-sixteenths, any two V-shaped dyads six-sixteenths, any three in four-sixteenths. As the one hundred spermatocytes counted represent two hundred derivative cells, we have:

	Expected	*Actual count*
A given V (Accessory or no. 1)	$\frac{1}{2} \times 200 = 100$	100
Only one V	$\frac{1}{4} \times 200 = 50$	48
Two given V's (Accessory and no. 1 or those of 7 and 8)	$\frac{1}{4} \times 200 = 50$	46 and 47
Any two V's	$\frac{3}{8} \times 200 = 75$	84
Three given V's (nos. 1, 7, 8 or accessory, 7, 8)	$\frac{1}{8} \times 200 = 25$	22 and 21
Any three V's	$\frac{1}{4} \times 200 = 50$	48
All four V-shaped	$\frac{1}{16} \times 200 = 12\frac{1}{2}$	8

Considering that these figures are based on only one hundred first spermatocytes, the results are probably as near the expectations as could be anticipated for any objects on the basis of chance distribution for a like number of trials.

b. Recombination of the homologues of the J-shaped tetrads in eighty-two individuals of Trimerotropis (?) fallax (?). The use of the word recombination may require some explanation. The two dissimilar homologues composing a heteromorphic tetrad are segregated into different male gametes. For example the atelomitic dyad of a J-shaped tetrad goes to one pole and the telomitic dyad to the opposite pole. Gametes carrying each kind of homologue would therefore be formed. If we may assume that the form of the homologues remains constant and that a female may carry J-shaped (heteromorphic) tetrads and produce different types of gametes, in respect to such chromosomes, like those of the male; then, if free fertilization occurs, there should be found in the offspring all possible recombinations of the

homologues; e.g., tetrads with two atelomitic dyads and tetrads with two telomitic dyads as well as J-shaped tetrads. The only way to demonstrate that such recombination occurs is through breeding experiments. But since I have collected at random a considerable number of individuals (eighty-two) and have analyzed their first spermatocyte complexes, and have found the types of chromosomes which would be expected from the above assumptions, I have ventured to use the term recombination to express the relationship of those different types to each other.

It is obvious from the above section, that the two individuals there considered formed sixteen sorts of spermatozoa, so far as the selected elements are concerned. A study of a comparatively large number of animals was undertaken for the purpose of finding, in the first place, whether or not the complex is constant for the species and, secondly, if not, to determine the range of variation. The results from sixty-two individuals are given in plates 2 to 9. The chromosomes on these plates are arranged in the same manner as on plate 1, except that only one complex from an individual is shown; therefore each of the horizontal rows represents one animal. There are two exceptions to this statement: number $32a'$ is a spermatogonial metaphase from the same individual as number 32, and number $63a'$ is a spermatogonial metaphase from animal number 63.

It will be seen from plate 1 that animal number 1 has, as already stated, in addition to the three J-shaped tetrads, four atelomitic and four telomitic chromosomes. Such a complex was found to be constant for two individuals. This statement is based on one hundred camera lucida drawings. Only one complex is shown from the succeeding animals, but a comparative study of a number of complexes was made in each case so that it can be safely stated that the complex is practically constant in any one individual.

Taking up animal number 2 (plate 2), we find five atelomitic tetrads, numbers 7, 9, 10, 11 and 12; one heteromorphic tetrad, number 1; and the remaining five telomitic. Passing on to animal number 3 we find six atelomitic, three heteromorphic (J-shaped) and only two telomitic tetrads. Chromosome number 1

which in the preceding animals has been heteromorphic has both homologues of the atelomitic type. If we study individual number 6 we find that it has no J-shaped tetrads; it has eight atelomitic and only three telomitic tetrads. Comparing individuals 6 and 7 it is seen that chromosomes number 9 to 6 inclusive are atelomitic in the former and J-shaped in the latter.

It is also interesting to compare more in detail individuals number 2 and number 6. Chromosome number 1 in individual number 6 is the type which would be expected to result if the spermatozoon carrying the V-shaped dyad of this chromosome in animal number 2, for instance, united with an egg bearing a similarly shaped dyad. The shape of chromosomes number 6 and number 8 of animal number 6 may be accounted for on a similar assumption. Conversely we would expect to find, then, in some individual the two telomitic homologues of these two tetrads, and this is exactly what we have in animal number 2.

Clearly, then, the complex is not constant in this species, according to our past conceptions of constancy as indicated by form of chromosome, since a given chromosome in one member of this species may be of the Stenobothrus type, in another of the Hippiscus type, while in a third, one homologue of this same chromosome is of the Stenobothrus type and the other of the Hippiscus type. This is most easily demonstrated by following chromosome number 1 through several plates. Taking plates 2 and 3 we find this element of the mixed type in eight of the sixteen individuals, while it is of the Stenobothrus type in four and of the Hippiscus type in four. For the entire sixty-two individuals under consideration this tetrad is of the Stenobothrus type thirteen times, Hippiscus type eighteen times, one homologue of each type thirty-one times. This comes very close to the 1-2-1 ratio that we might expect on Mendelian principles if the two types occur with equal frequency in nature and chance fertilization occurs.

The next point was to determine, so far as my material permitted, the range of variation. I wish to emphasize the statement previously made that the arrangement in the plates ac-

cording to size is only a rough estimate. Numbers 1, 2 and 4, I feel sure, are correctly identified in all cases, but aside from these there are scarcely any two consecutive ones which might not exchange places, yet the extremes are clearly distinguished. As the chromosomes are arranged, numbers 10, 11 and 12 are rings of the Stenobothrus type in all cases. I am inclined to think that this is correct. However, chromosome number 9 of the fifth animal might well change places with number 11. I have not been able to estimate satisfactorily the quantity of chromatin in the rings. On the other hand, it is readily seen on the slides that a given chromosome may be stretched out considerably with a comparatively slight thinning to compensate. The difference between an early and a late metaphase is also very striking. In the former the chromosomes are short and thick while in the latter they are evidently coming under some force which tends to stretch them out along the spindle axis. There is some evidence that this force is exerted through the spindle fibers; for instance, the accessory in individual number 45 (plate 7) has numerous fibers from both poles attached to it and its appearance certainly indicates that force is being exerted at the points of attachment.

It will be noted from the foregoing that any J-shaped tetrad has the capability of appearing in other individuals as a chromosome of either the Stenobothrus or Hippiscus type. Using this knowledge as a key, it is evident from a study of animals number 3 and number 32 (plates 2 and 5) that all of the chromosomes except number 2 and number 3 possess the potentiality of being of the Stenobothrus type in other individuals. If we then follow chromosomes number 2 and number 3 through the plates, we find chromosome number 3 in individual 16 (plate 3) to be a J, and in individual 21 (plate 4) we get the combination of two atelomitic homologues giving a Stenobothrus type of tetrad. Again, in individual number 31 (plate 5) it is a J. It is also of this form in animals number 7 (plate 2) and number 22 (plate 4). Chromosome number 2 does not occur except as a tetrad of the Hippiscus type in my collection. There was some fear that I might be confusing chromosomes number 3 and

number 5 because of their similar size and because not more than one in any individual varied from the Hippiscus form until I reached individual number 31 (plate 5). Here all of the chromosomes are either of the Stenobothrus type or J's except numbers 1 and 2 and they are too small for any confusion. Number 1 is freely of either type, so that we should expect to find, if a sufficient number of animals were studied, some with ten tetrads of the Stenobothrus type. The nearest approach to this possibility in these sixty-two specimens is the occurrence of eight in individual number 6 (plate 2). On the other extreme, it has already been noted that three rings and the accessory are atelomitic throughout. This would leave a possibility of eight telomitic tetrads. Individual number 62 (plate 9) is of this composition.

Plates 2 to 5 present quite a different appearance from plates 6 to 9, for the former group has many more chromosomes with nonterminal fiber attachment than the latter. The results are recorded in the order in which the animals were collected, which was also the order in which they were studied. The individuals represented on plates 2 to 5 were taken in the Yosemite Valley; Sisson, Cal.; and Eugene, Ore. Those represented on plates 6 to 9 are from Friday Harbor and Olga, Wash.; La Grande, Ore.; and Pocatello, Ida. They grouped themselves as shown without the slightest rearrangement. I refer to the first group as form A and to the second as form B.

The following table, made up from the plates, gives the total number of atelomitic and J-shaped tetrads. The first and third columns represent form A and the second and fourth columns represent form B. Each horizontal row, therefore, represents two individuals. The first horizontal row represents individual number 2 (plate 2), form A, in columns 1 and 3, and individual number 33 (plate 6), form B, in columns 2 and 4. The successive horizontal rows represent successive individuals in the two forms, i.e., numbers 3 form A, and 34 form B in the second row, numbers 4 form A, and 35 form B, in the third row, etc.

The ratio of atelomitic to J-shaped tetrads in the case of form A is 0.42; in the case of form B, 0.47. One might have expected in place of this relatively constant relation that the larger num-

NUMBER ATELOMITIC TETRADS	NUMBER ATELOMITIC TETRADS	NUMBER OF J TETRADS	NUMBER OF J TETRADS
Form A	*Form B*	*Form A*	*Form B*
5	4	1	1
6	4	3	2
5	4	4	2
5	3	3	1
8	3	0	1
4	4	5	1
5	3	2	2
7	3	2	2
5	4	2	1
6	4	1	1
4	3	4	2
5	4	1	2
4	4	4	2
6	4	1	1
6	5	2	2
6	3	2	4
6	4	3	2
3	3	5	1
6	3	2	2
7	4	1	3
7	4	2	2
5	3	3	1
5	4	3	2
4	4	3	1
6	3	1	3
6	4	2	1
3	3	4	2
6	3	1	5
7	4	1	1
6	3	3	6
5	3	4	1
Average....5.77	3.6	2.4	1.7

ber of Stenobothrus rings in form A would be compensated for by a smaller number of J's, or heteromorphic forms and the smaller number of Stenobothrus rings in form B by a larger number of J's. This might have indicated that one form was more viable in a given environment than the other. What we have is a uniform decrease in both Stenobothrus rings and J's in form B and a compensatory increase in the number of telomitic tetrads. The most important point to be noted is that if we may assume that heteromorphism is an indication of heterozygosity, then form

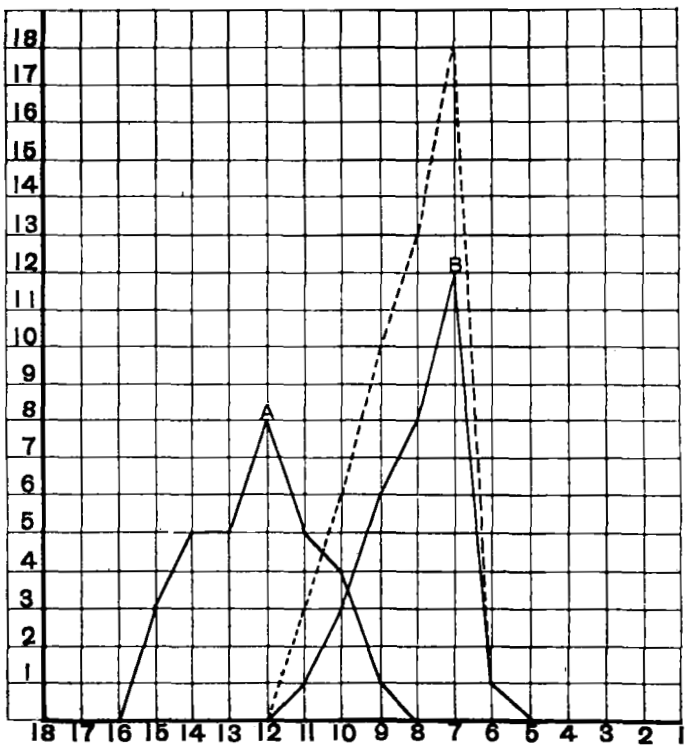
B is only about one-half as heterozygous as form A and consequently may be expected to be less variable taxonomically. That it really is less variable is indicated by the fact that taxonomists have separated the individuals that largely compose form A into four species, while they have treated the group that chiefly composes form B as a single species.

Since chromosome number 1 is the only tetrad subject to heteromorphism which could be recognized without possibility of confusion, its composition was studied in the two groups. Its behavior in the whole collection has been described on page 458. It occurs as follows:

	ATELOMITIC	J-SHAPED	TELOMITIC
Form A	8	16	7
Form B	6	16	9

In both cases it approximates closely a 1-2-1 ratio as it did for the entire collection. While it is evident then, that a rather general change has taken place in the two forms in respect to the number of atelomitic and telomitic chromosomes, it has not affected chromosome number 1. Likewise, a glance through the plates will show that the accessory (no. 4) and chromosome number 2 have remained constant. The remaining nine tetrads intergrade in size so closely that it is impossible to identify any given one with certainty. It was, therefore, necessary to study them collectively. The number of atelomitic dyads per individual was determined and the results plotted. The curves thus derived are shown in text figure 1. Numbers 10, 11 and 12 have remained constantly atelomitic, if I have been correct in my identification of them. But on the chance that I may not have been able to identify these correctly I shall include them in the curves; if their form is constant, they will not affect the shape of the graphs, while, on the other hand, there is a possibility that they are concerned in the changes. In text figure 1 the ordinates represent the number of animals; the abscissae the number of atelomitic dyads per cell. That is, each Stenobothrus ring is made up of a pair of atelomitic dyads, while the J-shaped tetrads have only

one. For example, individual number 3 has five Stenobothrus rings and three J-shaped tetrads making thirteen V-shaped dyads derived from the tetrads in question—that is, not including numbers 4, 2 or 1. We have then in these nine (fig. 1) tetrads, which



Text-fig. 1 Curves showing the number of atelomitic dyads, exclusive of chromosomes number 1, 2 and 4. The abscissae represent the number of such dyads, the ordinates the number of individuals, the broken curve includes data from twenty additional animals of form 'B.'

are concerned in the changes, the possibility of eighteen atelomitic dyads. The two solid curves each represent the same number of individuals, thirty-one, the total number in the collection of form 'A'. There were twenty more animals of form 'B' which have been studied but not included in the plates, since

they show no exceptional conditions. Data from these twenty are used in the broken curve of form B, which includes all of the specimens of this form in my collection.

Analysing these curves we see that in form A the extreme numbers of atelomitic dyads are 15 and 9, the mode 12 and the mean 12.3. In form B the extremes are 11 and 6, mode 7 and mean 8. It will be noted that the extremes in neither group reach to the mode of the other. The graph of form A is well balanced, indicating a stable group with considerable range of variation. That of form B, while showing much less variation, is skewed to the left, which would lead one to suspect that this group is changing towards the more stable form.

Plates 6 to 9 show chromosomes number 3 and number 5 always of the Hippiscus type in form B, though both are subject to change in form A. There is some question as to whether chromosome number 5 is always correctly identified. However, it is clear from animal number 60, which has three rings of the Stenobothrus type and five J-shaped tetrads, that eight tetrads have the potentiality of being atelomitic. It is puzzling that five (animal no. 47) is the nearest approach to this extreme found. Animal number 60 with its possibility for eight Stenobothrus rings offers one of the strongest indications that these two groups may be really members of a single species.

c. Segregation and recombination of homologues of J-shaped tetrads in eleven individuals of Circotettix lobatus. Eleven specimens of this species were taken in a very circumscribed area at La Grande, Ore. and a single specimen of another species (rabula) at Ogden, Utah. This latter animal had both telomitic and atelomitic chromosomes, but no J-shaped tetrads. This is not significant, however, as three of the eleven individuals of lobatus also lack J-shaped tetrads.

First spermatocyte complexes of eight of the eleven lobatus are shown (plate 10). The other three are included in the description. As just stated, three (or over one-fourth) of these animals have no J-shaped tetrads. Four have one and the remaining four have two. As in Trimerotropis, their dyads segregate at random in regard to each other and to the accessory.

The minimum of atelomitic tetrads in this limited collection

is four (animal no. 64, plate 10) and the maximum six (animals no. 69 and no. 71). Animal number 67 has, in addition to five telomitic tetrads, two J-shaped tetrads, exclusive of chromosome number 1, which in this instance is rod shaped, but which we see from other individuals may be atelomitic. In a large group of animals, then, one would expect to find a maximum of eight atelomitic tetrads. This species agrees in this respect with form 'B' described above. Chromosome number 1 is of especial interest; in four cases, it is of the Stenobothrus type, in three, rod-shaped, and in three, J-shaped, while in one animal (no. 70) it is extremely unequal. I will have more to say later concerning this point. Segregation and recombination of the dyads of the heteromorphic tetrads in this species does not differ from that in the larger group.

d. Origin of a tetrad with unequal dyads. Tetrad number 1, animal number 70, as mentioned above, divides very unequally as shown on plate 10. Furthermore, the fiber attachment is not terminal on the smaller part, which in size suggested at once that it was the free arm of the atelomitic dyad of the heteromorphic form of this tetrad. A study of the late prophases gave the key to the solution of the problem as to what might cause such an inequality. Here we find a tetrad, normal to all appearances, except that a pair of chromomere vesicles,[2] (so called plasmasomes) described by the writer in 1913, is attached near the middle of one dyad (fig. 70*d*, plate 11). Wenrich ('16) identified

[2] It seems desirable to qualify the term vesicle which I used ('13) to designate these structures as chromomere vesicles or vesicular chromomeres, as the case may be, in order to prevent confusion with the vesicular chromosome or chromosomic vesicles first described for the Orthoptera by Sutton ('00).

The suggestion comes that they may stand in somewhat the same relation to certain chromomeres as the chromosomic vesicles do to the chromosomes. Just as given chromosomes (e.g., the accessory) have a more marked tendency than others to become vesicular, these specialized chromomeres of different chromosomes may vary in this power. For example a chromomere, or granule, may merely become expanded with the chromatin more or less equally distributed as Wenrich ('16) has shown for one of the terminal granules of tetrad 'A' of Phrynotettix. Such an expanded chromomere may or may not go a step further and form a definite membrane giving a true vesicle; e.g., one of the terminal chromomeres of tetrad 'B' in Phrynotettix.

these vesicles as expanded conditions of certain granules, terminal in the case of his tetrads 'A' and 'B'. We also know from from the work of Pinney ('09) that the point of fiber attachment is usually marked by prominent granules. It, therefore, seems probable, especially since the other end lacks them, that the vesicles mark the point of fiber attachment on what should have been a J-shaped tetrad and that their formation has weakened the dyad with which they are associated to such an extent that division in metaphase occurs at this point. The result is that one and a half dyads go to one pole, the remaining half dyad to the other. This peculiarity seems to be constant for this animal. Figure 38*a* (plate 11) represents a similar possible origin for an unequal pair in one of the larger chromosomes in form B. This was the only instance of the kind found in this animal.

e. Other forms of heteromorphism. Two other types of heteromorphism remain to be considered. The first—a peculiarity common to both these genera—is a constriction of certain dyads of chromosomes number 3 and number 5. The behavior of tetrad number 5 in different individuals is shown in plate 14. Figures 21*a* and 13*a* are homomorphic, constricted and smooth forms, respectively. Figure 62*f* is the heteromorphic form. The same thing is shown in chromosome number 3 (plate 2), animal number 6 being homomorphic for the constricted form, number 2 homomorphic for the smooth rod and number 8 heteromorphic. Tetrads marked by this peculiarity are almost as striking as the J-shaped tetrads. And since chromosome number 5, frequently, and number 3, occasionally, are J-shaped or even of the Stenobothrus type, the J-shaped tetrads may have the telomitic dyads of the constricted form (plate 3, 16, chromosome no. 3); or of the usual smooth rod form. In other words, for these two chromosomes, there are three types of homologues: atelomitic, telomitic-smooth and telomitic-constricted.

The third type of heteromorphism affects chromosomes number 9 and number 11 as they are arranged on the plates. Both homologues are atelomitic, but there has been a secondary shifting of the point of fiber attachment so that the free arm of one dyad is much longer than the free arm of the other. The point

of union of the homologues is correspondingly nearer the bend in the dyad with the long free arm. Photomicrographs of these conditions are shown, figures 22*c* and 22*d* (plate 14). As indicated by the numbers, these two chromosomes occur in the same individual (plate 4, 22). They are not modifications of the same chromosome, for in numerous instances both are present in a single section of a cell. Figure 23*a*, from another specimen, shows a tetrad which is homomorphic for the long-armed condition of the homologous chromosome shown in figure 22*c*, while figure 10*a* is probably the homomorphic short-armed form of the same chromosome from another specimen. At least, this latter individual is homomorphic for the short-arms as may be seen from the drawing of the entire complex (plate 3, 10, chromosome no. 11). The homomorphic forms of tetrad number 9, shown in 22*d*, are represented in figures 17*a* and 10*b* (plate 14). Chromosome number 9 may also be J-shaped (plate 2, 7) or telomitic (plate 4, 25).

All possible combinations of the dyads in these two types of heteromorphic tetrads occur and segregate freely in relation to sex.

In the two heteromorphic types described above, as in the case of chromosome 'C' in Phrynotettix (Wenrich '16), we have a visible mechanism whose behavior in the maturation divisions corresponds to the segregation of triple allelomorphs.

f. Reduced number of chromosomes in Circotettix. Circotettix is the only genus of the Oedipodinae, so far investigated, to have less than the typical number of chromosomes. Numerous counts showed eleven to be the number in the first spermatocyte and twenty-one in the spermatogonia (plate 11, figs. 70*a* and 75). I am not satisfied as to whether the missing chromosome is number 2 or number 3. Plate 10 is arranged to emphasize its absence. The figures are from Circotettix lobatus, but rabula agrees in this respect. It is idle at present to speculate as to whether the chromatin which would have gone to form the missing tetrad has disappeared from the complex or whether it has united with some other tetrad to form an octad such as Robertson ('16) postulates to account for the reduced number in

Stenobothrus. This latter genus has but nine chromosomes in the reduced series (seventeen in spermatogonia). Three of these, however, are rings which present a typically different appearance from the remaining five tetrads. The basis of this difference is that they have entered the spindle extended parallel to its axis and have nonterminal fiber attachment. Robertson holds that these three chromosomes are really multiples; their point of union being represented by the position of the spindle fiber attachment. This would give twelve (the number typical for the Acrididae) as the real number of chromosomes in the haploid series. Woolsey ('15) has clearly shown such a linkage of chromosomes to be correlated with a reduction in number in several species of a Locustid genus. Furthermore, McClung ('16) has found conditions similar to those discribed by Miss Woolsey among the individuals of a single species of Hesperotettix.

The difficulty is, that Robertson puts forward a hypothesis that nearly all rings of the Stenobothrus type are multiples and are correlated with a reduction in the number of chromosomes. This is clearly not the case as regards either Trimerotropis (forms A and B) or Circotettix, for both have numerous tetrads of the Stenobothrus type and Trimerotropis has the normal number of chromosomes (twelve) while Circotettix has but one less. The number of rings of the Stenobothrus type varies from individual to individual, but the number of chromosomes is constant. In addition, the transition stage (J-shaped tetrad) between chromosomes of the Stenobothrus type and those of the Hippiscus type is present in these species. A further argument against the above hypothesis is the secondary shifting of the point of fiber attachment on certain chromosomes (figs. 22*c* and 22*d*, plate 14).

It is clear that we have two fundamentally different types of rings which are so similar morphologically that unless we get transition stages, as Woolsey ('15) has in Jamaicana and McClung ('16) in Hesperotettix, we have no criterion by which to separate them. Both enter the metaphase extended parallel with the axis of the spindle, both have non-terminal spindle fiber attachment, both give double V's in anaphase and both may occur where there is reduction in number without all of them being

correlated with the reduction (Circotettix and the Stenobothrus-like form described by McClung, '14). Yet structurally one is an octad the other a tetrad. The octad has true terminal spindle fiber attachment, so far as the tetrads composing it are concerned, while the ring of the other type has real nonterminal spindle fiber attachment.

g. Supernumeraries. Another variation which has attracted attention in our collection of Acrididian material for the first time and which is relatively very abundant in the specimens collected during the summer of 1915, is the presence in certain individuals of one, or sometimes two, entities which I shall designate as supernumeraries. They possess the staining capacity of chromatin. In the spermatogonia they enter the spindle at metaphase and divide like any chromosome (fig. 62*a*, chromosome *s*, plate 11 shows this body in the spermatogonia). In the plates of the first spermatocytes these bodies have been placed to the left of chromosome number 12, and a glance through plates 2 to 10 will show their frequency of occurrence. Individual number 10 (plate 2) of form 'A' possessed two supernumerary chromosomes, one more than twice the size of the other. This was the only animal in this group which possessed supernumeraries. But when we pass to form 'B' (plates 6-9) we find them in eight out of thirty-one individuals, that is, in more than one-fourth of all the animals studied.

I would especially call attention to the similarity between the supernumerary and accessory in specimen number 62.

The suprenumeraries are also present in two of the eleven Circotettix studied. It is evident from the plates that these elements vary greatly in size; when two are present in a given individual there may be very little resemblance between them. At the first spermatocyte division they pass to one pole undivided. When two are present they segregate freely in relation to the accessory and to each other. At the second spermatocyte division they again enter the spindle, at least usually, and divide as in the spermatogonia (plate 13 62*e*).

2. Spermatogonial metaphases and female somatic metaphases

a. Individual number 1. Dr. McClung ('14) has shown that generally the point of spindle fiber attachment is constant in all generations (spermatogonia, first and second spermatocytes). Therefore, knowing the number of dyads in the first spermatocyte with nonterminal fiber attachment, one would expect to find the same number in the spermatogonia. The animal represented on plate 1 has four Stenobothrus rings, each made up of a pair of dyads with nonterminal fiber attachment or eight such dyads in all; in addition, there are the atelomitic dyads of the three J-shaped tetrads and the accessory; so that we should expect twelve atelomitic chromosomes in the spermatogonia of this animal. Figure 1, plate 11, a spermatogonial complex from this specimen, shows the twelve atelomitic chromosomes. Entire complexes of spermatogonia were drawn in order to be certain that all of the chromosomes with nonterminal fiber attachment might be present. The telomitic chromosomes appear in outline only, as I wish to make the enumeration of the others as easy as possible.

b. Extremes found in the group. In order to show clearly the correspondence between the spermatogonial chromosomes and those of the first spermatocytes, two spermatogonial complexes, one from Form 'A', the other from Form 'B', have been rearranged so that the homologues are paired as they are in the first spermatocyte anaphase (with which they are compared) from the same individual. One of these spermatogonial complexes is shown with the chromosomes in their normal position in figure 32*a* (plate 11). The rearrangement of this complex may be seen on plate 5, figure 32*a*. Fifteen atelomitic dyads are present in both spermatogonium and first spermatocyte. Figures 36 and 63*a'* (plate 9) show similar arrangements of first spermatocyte anaphase and spermatogonial complex, respectively. Each has eight atelomitic chromosomes. Figure 63*a'* is a rearrangement of 63*a* (plate 11).

From the analysis of the first spermatocyte metaphases, we would expect animal number 62 to have the least number of atelomitic spermatogonial chromosomes, seven. This is seen

to be the case in figure 62*a* (plate 11). Information derived from the same source would lead us to expect seventeen atelomitic chromosomes in individual number 22. Such a metaphase is shown on plate 11, figure 22*a*. Figures 62*a* and 62*b* are spermatogonial metaphases from the same animal and have seven atelomitic chromosomes, as we would expect from the first spermatocyte complex of this individual (plate 9, 62).

Figures 70*a* and 75 (plate 11) are spermatogonial complexes from Circotettix lobatus. The significant difference from similar complexes of Trimerotropis is that they have twenty-one instead of twenty-three chromosomes. In regard to the atelomitic members the conditions are the same as in Trimerotropis, that is, the number of such dyads is the same in both spermatogonial and first spermatocyte metaphases. The number is twelve in these two cases. Figure 70*a* is from the individual in which tetrad number 1 divides very unequally in the first spermatocyte. It will be seen from this figure that the smallest two homologues are equal in size but one has median a fiber attachment. This agrees with the expectations from the study of the prophases of the first spermatocyte which showed a normal tetrad. We have then in the tetrad which divides unequally in the first spermatocyte, in reality a J-shaped tetrad which is dividing at the point of fiber attachment in the atelomitic dyad.

c. Female somatic complexes. Unfortunately, the maturation stages in the female are inaccessible because they do not take place until a few hours before or even after the egg is deposited (Henking '91), and the great quantity of yolk present has so far defied all efforts at sectioning. Immature ovaries are readily sectioned and contain numerous divisions in the follicular cells. These have been used for the somatic complexes. Morrill ('10) found from a study of the cleavage and early blastoderm stages of some Coreid Hemiptera that the number and size relations of the chromosomes in the somatic cells of the males and females are the same as in the spermatogonia and oogonia, respectively. Three females were studied by me, the complex proving constant for the individual, but

varying from animal to animal. Specimen number 74 with its fourteen atelomitic chromosomes (plate 11, 74) was taken in the Yosemite Valley, hence should belong to the group having the larger number. The other two are from Puget Sound, where we would expect the smaller number. One has ten (plate 11, 72) and the other, much to my surprise, has eight (plate 11, 73), which, if my conclusion that three of the atelomitic rings are constant is correct, would be the minimum number for the female, since she possesses two accessories, giving a complex of twenty-four chromosomes instead of twenty-three as in the male.

We may now answer the third and forth questions propounded at the beginning of this paper. The complex is constant for any given individual but varies within fixed limits for the species. Hence there is no need to assume any regulatory mechanism during maturation or fertilization.

3. Second spermatocytes

a. Types found in individual number 1. As has already been noted, the formula, $2^n_{..}$, in which n represents the number of pairs of chromosomes under consideration, gives the possible combinations in the gametes. According to this formula animal number 1, as we have seen on page 455, would be expected to form sixteen sorts of second spermatocytes. However, as we can not distinguish between chromosomes 7 and 8, we can identify only twelve sorts, as shown on plate 12. Numerically, they fall into two classes, those with eleven (figs. 1 *j* to *o*) and those with twelve chromosomes (figs. *p* to *u*), owing to the presence or absence of the accessory. In the class with eleven chromosomes we would always have the four atelomitic chromosomes derived from the four Stenobothrus rings (fig. 1 *j*). In addition we may have one of the larger atelomitic dyads (fig. *k*), the small atelomitic dyad (fig. *l*), the small atelomitic dyad and one of the large ones (fig. *m*), both large ones without the small (fig. *n*), or the atelomitic dyads of all three (fig. *o*). Figures *p* to *u* present a similar series, except that they each have one more atelomitic dyad, the accessory.

b. Extremes found in the group. Two individuals, number 6 (plate 2) and number 62 (plate 9), have no J-shaped tetrads and therefore would have only the two recognizably different sorts of second spermatocytes determined by the presence or absence of the accessory. Number 7 (plate 2) and 60 (plate 9), on the other hand, each have five J-shaped tetrads which, in combination with the accessory, would give 2^6 or sixty-four different sorts of spermatozoa. The distribution of the J-shaped tetrads in forms 'A' and 'B' as well as in the whole group, is shown by the curves (text-fig. 2). The ordinates represent number of individuals, the abscissae the classes of spermatozoa formed. The lower broken curve represents form 'A', the solid line form 'B', while the upper broken line is a composite of the two. It is interesting to note that while the extremes of both forms are the same and contain the same number of animals, there are only four of the thirty-one members of form 'B' which give more than eight kinds of spermatozoa, while fifteen, or practically half, of the members of form 'A' give sixteen or more kinds.

Individual number 62 is of interest on account of the large supernumerary present in the spermatogonia and first spermatocytes. There are no J-shaped tetrads in this specimen, but it is possible to recognize four sorts of second spermatocytes. Of two sorts with three atelomitic dyads one has eleven chromosomes and the other twelve—due to the presence of the supernumerary. Two sorts have four atelomitic dyads, i. e., the accessory in addition to the three derived from the three Stenobothrus rings of the first spermatocyte. One of these has the normal number (twelve) and the other thirteen, since the latter also contains the supernumerary.

Individual number 63 (plate 9) is one of the easiest to follow out, since it has only one J-shaped tetrad. This, with the accessory, gives two differential chromosomes, from the distribution of which we should expect 2^2 or four sorts of second spermatocytes. Considering all the atelomitic dyads, including those derived from the three Stenobothrus rings, we find four

morphologically different sorts of second spermatocytes as follows:

11 with 3 atelomitic dyads (fig. 63*d*)
11 with 4 atelomitic dyads (fig. 63*c*)
12 with 4 atelomitic dyads (fig. 63*b*)
12 with 5 atelomitic dyads (fig. 63*e*)

The transformation stages which result in the mature spermatozoa appear to be perfectly normal throughout the collection.

c. Circotettix. Circotettix does not differ from the species just described so far as the formation of different types of spermatids is concerned. But we have the important difference that the numbers are ten and eleven (barring the supernumeraries which are present in two of the specimens) instead of the usual eleven and twelve. Complexes from two individuals are shown in figures 69*a*, 69*b* and 70*b*, 70*c* (plate 13).

III. SUMMARY OF OBSERVATIONS

1. The number of spermatogonial chromosomes in Trimerotropis is twenty-three. Anywhere from seven to seventeen of these have been found to be atelomitic, but the number of such chromosomes is constant in a given individual.

2. The number of spermatogonial chromosomes in Circotettix is twenty-one. Nine to thirteen of these have been found to be atelomitic in different individuals. The number of atelomitic chromosomes is constant for the individual.

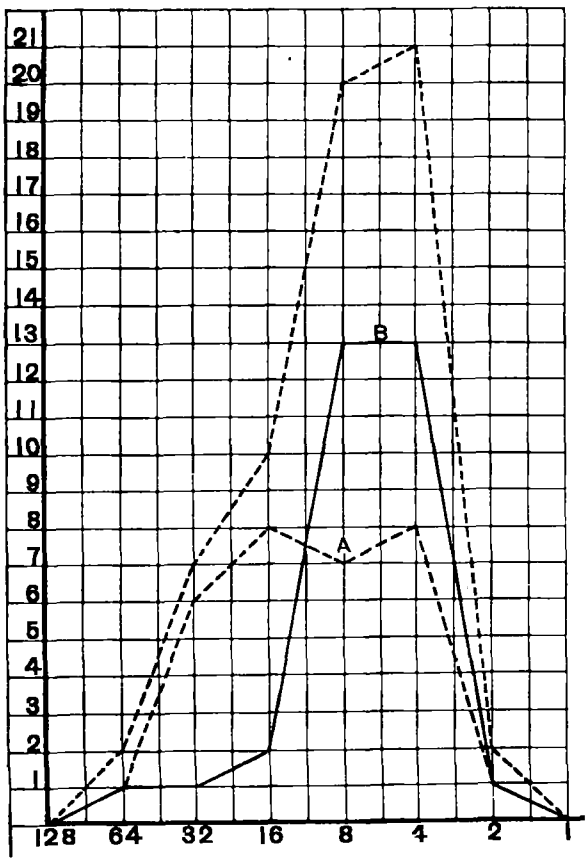
3. The number of somatic chromosomes in the female of Trimerotropis is twenty-four. The number of atelomitic chromosomes varies in different individuals from eight to fifteen, but is constant for the individual.

4. The number of chromosomes in the first spermatocyte of Trimerotropis fallax is twelve, of Circotettix eleven.

5. Certain individuals may possess one or two supernumerary chromosomes. These bodies divide in the spermatogonia, pass to one pole undivided in the first spermatocyte division and divide in the second spermatocyte. They segregate in the

first spermatocyte without relation to the accessory, or to each other when two are present.

6. The first spermatocyte tetrads show three types of heteromorphism: 1) one homologue telomitic, the other atelomitic,



Text-fig. 2 Curves showing the number of morphologically different classes of spermatozoa. The abscissae represent number of classes, the ordinates number of individuals. The large broken line is a composite of 'A' and 'B.'

giving J-shaped tetrads. 2) both homologues atelomitic, one with nearly median, the other with subterminal fiber attachment. 3) both homologues telomitic, one smooth, the other constricted; or, one telomitic constricted, the other atelomitic.

7. All of these types of heteromorphic homologues segregate at random in the first maturation division.

8. Considering the J-shaped tetrads alone, of sixty-two males, two formed sixty-four morphologically different sorts of spermatozoa, seven formed thirty-two sorts, ten formed sixteen, twenty formed eight, twenty-one formed four and only two were dimorphic.

9. The complex is constant in the individual for somatic, spermatogonial and first spermatocyte metaphases and has a fixed range of variation for the second spermatocytes. Certain exceptions to this general rule have been noted in the text, e.g., the accessory with numerous fibers attached (plate 7, 45) and the tetrad which divides unequally in Circotettix (plate 10, 70).

10. The complex varies widely in the group within the limits of the above mentioned types of heteromorphism, indicating, so far as my material goes, that random mating of the various classes of morphologically different gametes has occurred.

11. Taxonomically the group is exceedingly variable.

IV. DISCUSSION

1. Constancy in individuals

To me one of the most impressive facts in this whole work has been the very great degree of individual constancy of the complex which has been preserved in the organism in spite of the wide range of morphological variation between the homologous chromosomes in these species. It should be emphasized that the term individual constancy is applicable here, although it is used in a broader sense than is ordinarily the case; that is, while the somatic, spermatogonial and first spermatocyte complexes are practically identical for the respective generations in a given individual, there is a fixed number of morphologically different classes of second spermatocytes for each individual, but in all cases these can be calculated from a knowledge of the first spermatocyte complex.

It is futile to speculate at present on the cause of this heterogeneity, but so far as the doctrine of chromosome individuality

is concerned the manner of its origin is of minor importance. The essential thing is that it exists, and is transmitted, not only from cell generation to cell generation in the individual, but occurs so definitely that one can predict the combinations that should occur in the population. Judging from the conditions present in my material one would find all possible associations if a reasonably large number of specimens were examined.

Actual observation has shown the ratios of the telomitic to the atelomitic dyads of chromosome number 1 to be 1 to 1. In other words the variant, atelomitic homologue, occurs in practically one-half of the population. But we cannot generalize that this ratio holds, therefore, for all of the variants, if we may so designate all atelomitic dyads. Tetrad number three has only six atelomitic dyads out of a possible one hundred and twenty-four in the sixty-two individuals represented (plates 2 to 9). It is impossible to calculate ratios for the occurrence of any given combination in the general population unless one knows in advance the frequency of occurrence of that particular variation.

It is hard to imagine a better example of persistent individuality than that illustrated in figures 21*a*, 62*f*, 13*a*, plate 14 where a variation from the normal smooth rod form (fig. 13*a*), such as the constriction of the dyads (fig. 21*a*), has been transmitted, not only from cell to cell in one individual, but apparently on to the next generation, so that the union of a gamete carrying such a chromosome with one bearing its normal homologue has resulted in a zygote which shows both types clearly (fig. 62*f*). This is the same principle found throughout this work where no kind of heteromorphism (J-shaped tetrads and atelomitic tetrads with unequal arms) has been found without homomorphic forms corresponding to both types of dyads occurring in other individuals.

There are but two other instances in all cytological literature so far as I know where homologous chromosomes show morphological differences other than those of size. One of these is the multiple of Mermeria (McClung '05). The other case is that reported by Voïnov ('14). It seems desirable to undertake a

rather extensive review of the latter paper on account of some questionable conclusions and on account of some points of general similarity, as I believe, in our material.

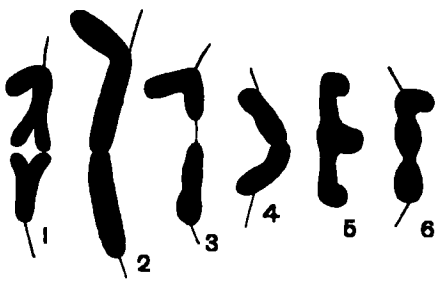
Voïnov is dealing with Gryllotalpa vulgaris. This is the form for which vom Rath ('92) reported the somatic number of chromosomes as twelve. He described a doubling of this number just before the first maturation division which reëstablished the normal number. The second division brought about reduction to one-half the normal. Vom Rath extended to all animals his conclusion that there is a doubling of the number of chromosomes before the maturation divisions, both of which are reductional and result in halving the normal number of chromosomes. As this erroneous idea appealed to many as a support of Weismann's hypothesis it was widely accepted for a time. Hence work on this form is of more than usual interest.

Voïnov figures fifteen chromosomes in the spermatogonia. The smallest of these is clearly without a morphological mate and he considers it to be in reality a pair of microchromosomes synapsed in the spermatogonia. This assumption leaves him without a *Y* for what he considers an *XY* pair which occurs in the first spermatocytes. He assumes the *Y* exists in the spermatogonia but has escaped detection. Therefore, he concludes that seventeen is really the somatic number, while he finds seven in both maturation divisions. This is accounted for by further assuming that two of the seven must be multiples; one an octad, the other a hexad. The hexad he believes to be identical in composition with the chromosome 'en L' of de Sinéty ('01) and the hexad of McClung ('05). A comparison of Voïnov's chromosome marked 'a' and 'bi' (text fig. 8*c*, see my text fig. 3, *3*) with de Sinéty's chromosome *c–s* (fig. 93, plate 3, see my text fig. 3, *1*) and with McClung's chromosome 'ac' (text fig. 3, see my text fig. 3, *2*) will show a critical difference. In both the latter, if we may assume that de Sinéty's chromosome 'en L' is a hexad, the tetrad part divides, resulting in a greater quantity of chromatin passing to one pole than the other, while Voïnov's 'hexad' divides in such a manner that, though the parts are morphologically different, the quantity of chromatin in each

would seem to be about the same. Voïnov recognizes this difference, he says (p. 474):

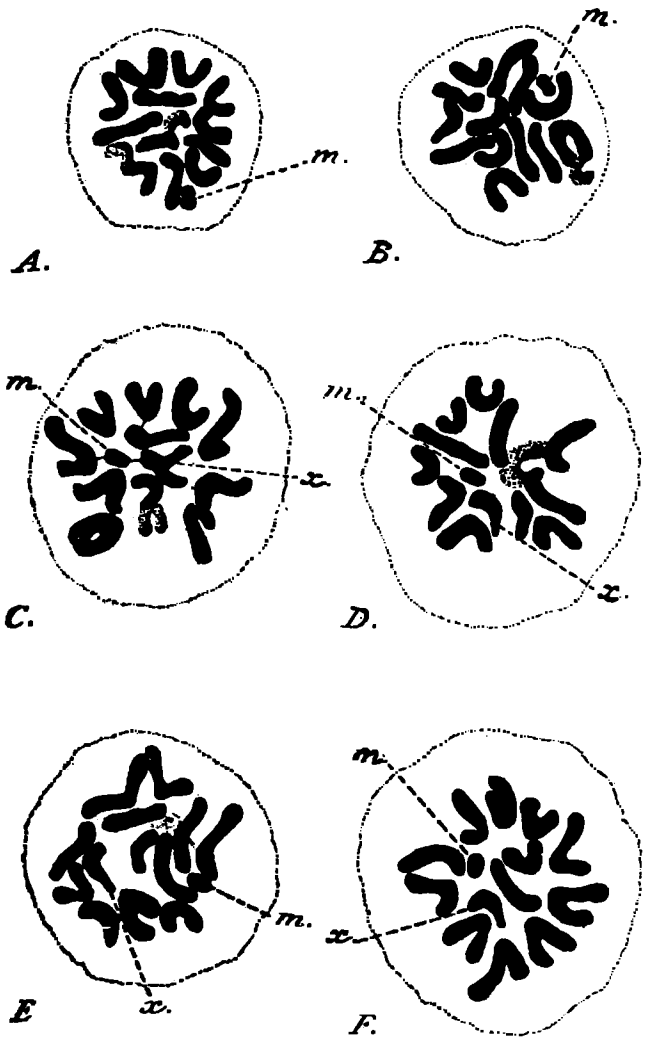
> Il s'ensuit que le chromosome accessoire est distribué seul à un pôle et le bivalent entier avec lequel il était associé va au pôle opposé. Ce bivalent donc, comme le chromosome accessoire, ne se divise pas dans la première mitose de maturation. Cette manière de se comporter constitue une différence avec les chromosomes multiples décrits par Sinéty et Clung.

That is, while its structure is the same, its behavior is different. It will be obvious to the reader that this chromosome



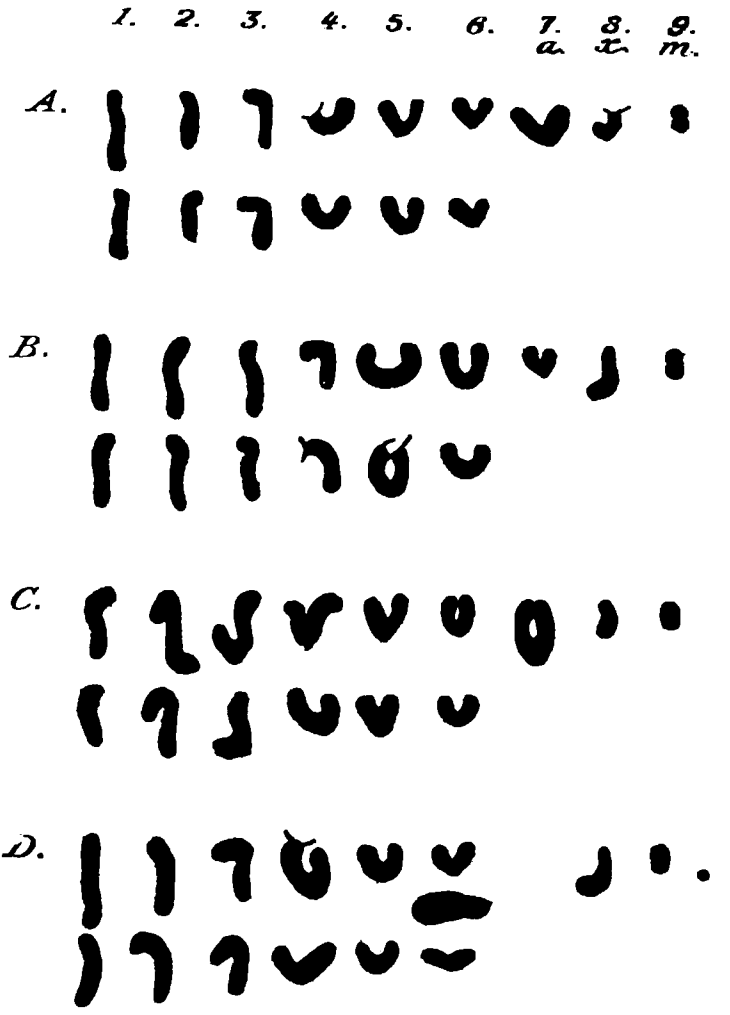
Text-fig. 3 1 'Chromosome en L,' de Sinéty, plate III, figure 93.
2 Hexad multiple, McClung, '05, p. 309, figure 3.
3 'Chromosome –L,' Voïnov, '14, p. 473, figure 8*c*.
4 Late second spermatocyte metaphase of 'l'element bivalent' Voïnov, p. 488, figure 16*c*.
5 'La grande tétrade,' Voïnov, plate XXIV, figure 32*t*.
6 'L'idiochromosome, XY,' Voïnov, Plate XXIV, figure 29.

corresponds both in form and in behavior in the individual with the J-shaped tetrads described in this paper. But, if it does correspond, one would expect certain individuals to contain in its place a rod-shaped tetrad and others a ring of the Stenobothrus type. That such a ring occurs in Voïnov's material may be seen in his figure 32*t*, plate 24, or my text figure 3, *5*. While he has material from fifty animals, it is impossible to judge how thoroughly it has been studied from the standpoint of the occurrence or non-occurrence of this 'hexad.' The only direct statement is in regard to a count to show the distribution of its parts in relation to those of an unequal pair. This



Text-fig. 4 A photographic reproduction of Voïnov's text-figure 1, showing that different individuals possess different numbers of V-shaped (atelomitic) chromosomes. A and B from the same individual show eight or nine. C from a different animal at least eleven, possibly thirteen. D from a third animal nine or ten. E and F from a fourth specimen have eleven.

count, he states, is based on a single individual. However, if my interpretation is correct, one should find varying numbers of atelomitic chromosomes in the spermatogonia of different individuals, while on Voïnov's statement the complex should



Text-fig. 5 A photographic reproduction of Voïnov's text-figure 2. The difference in the number of telomitic and atelomitic chromosomes possessed by individuals B and C is well shown.

be morphologically the same for all individuals. Voïnov shows (p. 448, fig. 1, see my text fig. 4) spermatogonial complexes from four individuals. *A* and *B* are from the same individual and agree in having eight or nine atelomitic chromosomes. *D*, from another individual, seems to be of the same type, while *C* and *F* from two other individuals, have eleven or twelve. This variation is made conspicuous by the arrangement in his text-figure 2 which shows strikingly the difference of the complex of individual *C* from that of *B*. However, Voïnov states (p. 450) that the form and size are constant for the species. To quote: "Dans les dessins où les chromosomes des quatre premières plaques sont groupés suivant leur resemblance (fig. 2), on voit que la forme et la grandeur sont constantes pour l'espéce." On the contrary, should one judge by these figures, (see my text-fig. 5) one would conclude that even the number was not constant since *D* lacks chromosome number 7. Also numbers 7A and 7B which purport to be homologous chromosomes,—drawn even from the same individual, —certainly bear no resemblance in size. This may better be attributed to the fault of the observer than to the material, as chromosome 7B would seem from the drawing to be much more homologous with 6A, and one member of 6B with 7A.

This evidence, together with the fact that none of his second spermatocyte figures show any indication of the presence of a tetrad [His chromosome 'b' (p. 488 *C*, see my text fig. 3, *4*) looks like any ordinary dyad and not a tetrad], has convinced me that his 'hexad' is a J-shaped tetrad. This would simplify matters, as on Voïnov's interpretation there are two sex-determining mechanisms present:—an accessory which forms part of the 'hexad' and in addition an unequal pair which he believes to be an *XY*. This leads him to remark (p. 496): "C'est un cas très rare, comme celui de Banasa calva parmi les Hémiptères, où de meme, grâce á la coexistence de deux chromosomes sexuels, naissent quatre catégories de spermatides, distinctes matériellement."

In 1905 Wilson reported the presence of an accessory and an idiochromosome pair in Banasa calva, but in 1907 he published

a note (which apparently Voïnov has not seen) in which he identifies as a supernumerary the element that he had formerly reported as the accessory.

Payne found what he believes to be a sex group in Gryllotalpa borealis. That is, an accessory and an unequal pair, the larger member of which always moves to the same pole as the accessory.

McClung has recently been able, through a knowledge of the J-shaped tetrads, to solve the puzzle of the multiple in Mermeria where he reported ('05), p. 316 that, "Entire tetrads pass into the second spermatocytes." It is now evident that the accessory is associated with a J-shaped tetrad which divides in the usual manner. Since apparently the accessory always attaches to the telomitic dyad, there would seem to be formed a sex-linked association comparable in end results with Payne's interpretation of conditions in Gryllotalpa borealis, the difference being that a physical union is lacking in the latter case. Voïnov's figures of his *XY* pair (plate 24, fig. 29, see my text fig. 3, *6*) strongly suggest the association of a third element with a normal tetrad; making this a true hexad multiple. A study of Wilson's figures of idiochromosome pairs suggests that they also might be hexad multiples; i.e., that the *X* is the accessory plus one dyad of a tetrad of which the homologue is the *Y*. Since we know tetrads composed of unequal homologues exist, there is no reason apparent why the accessory might not be associated with either the larger or smaller member. This might account for instances in Drosophila, where the *Y* is larger than the *X*. This explanation of the known facts would seem simpler than that of Wilson ('11), who attempted to derive all from a primitive *X*-*Y* pair.

It may be well to note the bearing of the evidence as to the time of segregation brought out by this work on Bateson's 'Reduplication hypothesis.' Bateson ('15) in a study of the sweet pea found coupling of two pairs of factors which he represents by the formulae A a and B b, giving the heterozygote, A a B b. The zygotic ratios obtained in F_2 showed that certain combinations between these factors occur more frequently than others. This led Bateson to postulate the theory that

the original zygotic cell divided into two similar cells, A a B b but that the second cleavage plane passed differently through these two cells so that one gave daughter cells A B and a b, the other A b and a B. Some of these four cells divided oftener than the rest so that more gametes of certain kinds were produced. As to the time of these segregation divisions he says (p. 299):

> Moreover the excess of gametes of parental composition characterizing the coupling- and repulsion-series must certainly mean that the position of the planes of division by which the four quadrants are constituted is determined with regard to the gametes taking part in fertilization—though the relative positions of the constituents of the cells may perhaps be maintained throughout the history of the tissues, it is easier to suppose that the original planes of embryonic division are determined according to those positions than that their influence can operate after complex somatic differentiation has been brought about.

Morgan ('15) has shown that the series of facts dealt with by Bateson is the same as those treated by Morgan and his students under the term linkage and that they are open to the same explanation.

It is evident that if such a somatic segregation as Bateson advocates occurred in Trimerotropis or Circotettix, we would have different types both of spermatogonia and of first spermatocytes in the individual, i.e. the metaphases would show varying numbers of atelomitic chromosomes, instead of being constant as I have found them to be.

2. Heteromorphism, supernumeraries and reduction in the number of chromosomes

The more I have studied this unusual group of grasshoppers the deeper has grown the impression that all of its peculiarities are closely interrelated. As was pointed out elsewhere (p. 465) there is some ground for associating the formation of vesicles with a shifting of the point of fiber attachment and also with a weakening of the chromosome at that point. In one animal, division constantly occurred at the point in question. It is

conceivable that this weakness might result merely in a constriction such as marks certain homologues. Figure 38 *a* (plate 11), where one of the larger tetrads in a member of form B is dividing at such a point, favors this interpretation. This is the only instance of this sort found in animal number 38.

So far, in all of our work, we have never found one of the larger tetrads with unequal homologues. On the other hand such peculiarities occur rather frequently among the smaller ones. This may indicate that the smaller chromosomes are less important. That they may be in part dispensed with was shown by Wenrich ('16) who finds that his tetrad 'C' lacks both terminal granules in certain individuals; and it seems possible that chromosome number 2 has been entirely eliminated from Circotettix. The homologues of the larger tetrads may be so important that a zygote lacking in even a part of one cannot develop.

The further suggestion presents itself that irregularities in mitoses such as have just been described, may be responsible for the occurrence of supernumerary chromosomes. Gametes containing a complete dyad plus a portion of its homologue would probably be viable and one may readily imagine that the extra portion might become detached in a succeeding division and might then form a supernumerary. Such an element would probably contain merely a reduplication of factor loci already present in the two dyads from whose homologue it was derived and might therefore have no influence in heredity.[3] On the other

[3] Dr. P. W. Whiting has suggested that it may be of interest to geneticists to point out that chromosomes such as numbers 7 and 8 (plate 1), which are indistinguishable morphologically, behave as duplicates, corresponding to Shull's theory of duplicate genes, and not as tetraploid homologues, as Muller has suggested for Gregory's tetraploid Primulas, e.g. If *A A'* equals the atelomitic dyads and *a a'* their telomitic homologues on Shull's theory *A* would always segregate from *a*, and *A'* from *a'*, or telomitic from atelomitic, giving a 1–2–1 gametic ratio. On the other hand, if they were tetraploid homologues in Muller's sense, *A* might segregate from *A'* and *a* from *a'* just as frequently, giving a 1-4-1 gametic ratio. In other words we would sometimes find the atelomitic dyad of chromosome number 7 mated with the corresponding one of number 8 and the same for the rod-shaped homologues. Since this condition does not occur, we must conclude that their morphological resemblance does not involve their behaving as tetraploids.

hand a supernumerary might contain the locus for multiple allelomorphs, in which case it might have an influence. The varying sizes found are perhaps due to two causes: (1) origin from different tetrads, (2) degeneration. More than one-fourth of the population of form 'B' contain supernumeraries, and, though one would expect that they would automatically pile up, no individual has been found with more than two. It therefore seems evident that there must be some method of elimination.

Wilson ('09) believes the supernumerary chromosomes in the Hemiptera are additional small idiochromosomes, and states that they are about the same size, of like behavior and show (Wilson, '07) some degree of coupling with the small idiochromosome. The supernumeraries found in the course of my work could not have had such an origin, since the sex-determining mechanism in the grasshopper is the accessory and not an X-Y pair. They are constant in size in a given individual, but range in different individuals from the size of the accessory (plate 9, 62) to less than a fourth of that size. In behavior during the growth period they simulate the accessory. If Wilson's hypothesis (that they are derived from the sex chromosome) were correct, my individual number 62, which appeared to be an entirely normal male, should have been a female; for the essential difference between the sexes is the possession of two accessories by the female, and in this particuar case the supernumerary, in the first spermatocyte metaphases simulates the accessory so closely both in size and appearance, that it is sometimes difficult to decide which is which. It seems probable that it was a large supernumerary of this type that Davis ('08) confused with the accessory when he described two 'monosomes' (accessories) as being present in one specimen of Arphia tenebrosa (p. 102).

3. Taxonomic variability and gametic composition of the group

My own slight knowledge of taxonomy, together with a recognition of similarity of habits and environment acquired through field work, enabled me to group these related forms as they were collected. The specimens were later turned over to

Mr. J. A. G. Rehn of the Academy of Natural Science for identification. My study of the germ cells was completed, the plates made and the curves plotted before I knew the result of Mr. Rehn's study. Similarly, Mr. Rehn classified the animals without knowledge of my results, or even of the localities from which the various specimens were taken. It is but fair to say that, owing to lack of time, all that Mr. Rehn attempted to do was to place the animals in the classification already established, although he hopes soon to undertake a revision of the group.

One of the most striking differences shown by the study of the germ cells is the apparent reduction in number of chromosomes in the form which is unquestionably Circotettix. There is no conflict here between taxonomic and cytological evidence. Two species come in this division, lobatus and rabula. Unfortunately, I have only a single member of the latter species and the present work has shown that any attempt to establish specific differences on one or two individuals is futile for this group.

The remaining eighty-three members of this group may be placed in two subdivisions, according to the number of atelomitic chromosomes in the duplex series as is shown in text figure 1 (page 463). The mode for one of these subdivisions is twelve, for the other seven. There is an overlapping of the two groups involving twenty individuals, ten from each form. But since the extremes of neither form reach to the mean of the other they might constitute two distinct species. However, since these groups are from widely separated localities, the differences may well be due to isolation. If we consider what the expected progeny of parents like number 60, form B, with its five J-shaped tetrads would be, it is evident that some of the offspring would be placed in form A according to this scheme.

It is interesting to note that all members of my form A were classified by Mr. Rehn as the Trimerotropis fallax of recent literature. On the other hand he placed three of the fifty-one members of my form B in this same species. The remaining forty-eight were identified as Circotettix suffusus as that species is at present recognized. He states in a note that this is a "Divergent Circotettix tending strongly towards Trimerotropis."

From my cytological studies, I do not hesitate to say that form B is a Trimerotropis, and furthermore, it is a question if Circotettix suffusus and Trimerotropis fallax, as they are now recognized, do not even constitute a single species.

4. Correlation of chromosomal behavior and Mendelian principles

The evidence pointing to the chromosomes as the bearers of the heredity determiners has been summarized so often recently that I shall not repeat the process. One of the chief difficulties that cytological research has met with has been the impossibility of distinguishing between the chromosomes derived from the two parents. While it has been clear that homologous chromosomes segregate into different gametes, it has been impossible to say, except in the cases of the tetrads composed of unequal dyads recently reported (Carothers '13, Wenrich '14, Robertson '15), that all of the chromosomes brought in by the egg do not pass into the female-producing spermatozoon, as was suggested by Payne ('09) as a result of his study of Gryllotalpa borealis.

Attempts have been made to determine the behavior of the paternal and maternal chromosomes in the maturation divisions and also to correlate given chromosomes with given somatic characters. Heretofore, the most promising line of attack on such problems has been through hybridization of forms with morphologically different chromosomes. The most noted of these experiments is that of Moenkhaus ('05). He crossed Fundulus heteroclitus and Menidia notata. The former has long, straight chromosomes, while those of the latter are short and curved. In the first few cleavages of the hybrid zygote, the chromosomes derived from one parent remained separate from those derived from the other. In later divisions the chromosomes became mixed on the spindle. But the important fact was demonstrated that the chromosomes derived from each parent maintained their morphological identity. The embryos did not develop beyond the closing of the blastopore.

Perhaps the most significant recent work along the line of hybridization is that of Federly ('13) who has combined breeding

and cytology in his study of the moth, Pygaera. He crossed P. anachoreta and P. curtula, and found by a study of the spermatogenesis of the hybrid, that pairing occurs between only two chromosomes; thus in the first spermatocyte of the parent forms there are twenty-nine or thirty chromosomes, while in those of the hybrid there are forty-eight of about one-half the size of the parental forms. When a back cross was made with one of the parent species, normal pairing took place between about one-half of the chromosomes of the hybrid and those of the parent species to which the cross was made, giving about thirty large paired chromosomes among a corresponding number of small unpaired ones. As one would expect from this cytological knowledge, both the primary hybrids and those resulting from the back cross were intermediate in all characters except one or two which showed normal dominance and segregation. The difficulty in working with such a form lies in the large number of chromosomes and in the lack of any means of distinguishing between them.

The work of Morgan and his students on Drosophila ampelophila is too well known to need much discussion. Here there is the advantage of a small number of chromosomes which differ in size and shape. According to Metz ('14) there are four pairs as follows: a pair of microchromosomes, a pair of sex chromosomes, equal in the female (XX), unequal in the male (XY), and two large V-shaped pairs.

Breeding tests with numerous mutants have shown one large group of genes, over thirty in number, to be sex-linked, hence borne, presumably, in the X chromosome. Two other great groups of over twenty members each, segregate independently and are assumed to correspond to the two large euchromosomes. Two characters so far—bent wings reported by Muller ('14) and eyeless, by Hoge ('15)—have been found whose genes segregate independently of the other three groups. Should mutations occur with equal freedom at any locus in the chromatin it would be expected, as pointed out by Muller, that the small microchromosomes would show fewer mutations than the larger chromosomes, and since the genes for bent wings and eyeless must lie

in one of the three pairs of euchromosomes, the presumption is that these two genes, rather than either of the two large groups of non-sex linked genes, lie in the microchromosomes. Should such prove to be the case, these would be the first instances of definite somatic characters being associated with a definite, recognizable euchromosome.

Dr. McClung, in two papers ('05 and '08), pointed out the general possibilities of cytological work and stated clearly his ultimate aim (p. 326, '05), "To determine the relation between individual chromosomes and characters in the body." For such a purpose the advantages of a species like Trimerotropis fallax or Circotettix lobatus is obvious. Taxonomically, there is striking individual variation, and correlated with this, apparently, are definite morphological variations, already described, of the homologous maternal and paternal chromosomes. Here, then, we have in a single species the means of distinguishing between certain homologous chromosomes. There is the further advantage of freedom from the sterility and lack of viability (if one may judge by the plentifulness of the animals) usually associated with hybrids. Of course it follows, that any ratios obtained through genetic work on these forms should be accurate, whereas those based on hybrids where there is a high degree of mortality, cannot be reliable.

So far no attempt has been made to breed these species, all of which, unfortunately for a worker located in the east, are Rocky Mountain forms. Another species of Circotettix occurs in mountainous regions of the east and, should it be as favorable cytological material as the western forms, I hope to do breeding work with it.

It might be said in passing that, according to my experience with several local species, grasshoppers are proving very favorable subjects for breeding. They stand captivity well and can accommodate themselves to various food and temperature conditions. They show strongly marked characters, breed freely, and it is possible to obtain three or four generations a year, at least of some species, by keeping them constantly at about 39°C. Special laboratory facilities, while convenient, are by no means essential since the eggs of most species normally live

through the winter out of doors and even the adults endure great extremes of temperature. The prime requisites for this work, aside from the 'hoppers' themselves, are fine wire cages and patience.

In any case, a study of a collection of individuals living in their normal environment was desirable, for, had such conditions first been found in captive stock, one would surely have suspected that these unusual conditions were due to the artificial environment.

This work has been sufficient, I believe, to show the random segregation of the homologues of all but four (nos. 2, 10, 11 and 12) of the first spermatocyte tetrads, and that the recombinations found in the group correspond to what would be expected according to the law of chance.

V. CONCLUSION

This material clearly presents the following facts:

1. Constancy of the chromosome complex for the individual.
2. The occurrence of heteromorphic tetrads in the first spermatocyte.
3. The segregation of these heteromorphic homologues in the first maturation division according to the law of chance.
4. The formation of the expected classes of second spermatocytes.
5. Normal transformation stages for the spermatids.
6. The occurrence in a group of individuals of practically all possible combinations of the heteromorphic chromosomes.

These facts taken together may be accounted for by:

1. The ordinary process of free, chance fertilization acting in a species in which the present forms of the chromosomes which originated through some past reorganization are stable, or:
2. A reorganization of the complex, involving a change of fiber attachment for each individual before the setting off of the germ cells.

Both of these possibilities may be tested by breeding:—In the first case parents of known chromosomal constitution would give progeny with a fixed range of variation—limited by the

number of possible combinations of the morphologically different gametes of the parents.

In the second case, any variation found in the group might occur in the offspring of a single pair.

The first explanation is a simple, logical one which meets all known facts; hence, in accordance with scientific custom, we are justified in accepting it for the present.

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EXPLANATION OF PLATES

The drawings were made with the aid of the camera lucida at a magnification of 2400 diameters and have been reduced one-third in reproduction.

Plates 1 to 10 show side views of first spermatocyte metaphases, arranged as nearly as possible according to size. Each horizontal row represents one cell, the vertical rows, corresponding chromosomes in different cells. They are so arranged that the accessory, number 4, is always passing to the upper pole.

The individuals from which drawings were made were numbered consecutively from one to seventy-five. The same number always applies to the same specimen; successive figures from any individual are indicated by letters.

When supernumerary chromosomes are present they are placed at the left of chromosome number 12 to indicate that they are out of the normal series and especially because they would occupy no definite position in relation to size.

Plate 14 is composed of photomicrographs made at a magnification of 1140 diameters and reproduced without reduction.

PLATE 1

EXPLANATION OF FIGURES

Lateral views of first spermatocyte complexes from a single individual (no. 1), showing mode of segregation of three heteromorphic tetrads (chromosomes no. 1, 7, and 8.

b to *e* Alternate distribution of atelomitic dyads of chromosomes number 7 and number 8.

f and *g* Concurrent distribution of same homologues segregating opposite the accessory. In *f* the atelomitic dyad of chromosome number one is accompanying the similar dyads of chromosomes number 7 and 8, while in *g* it is passing to the opposite pole.

h and *i* Same as *f* and *g* except that atelomitic dyads of numbers 7 and 8 are passing to the same pole as the accessory.

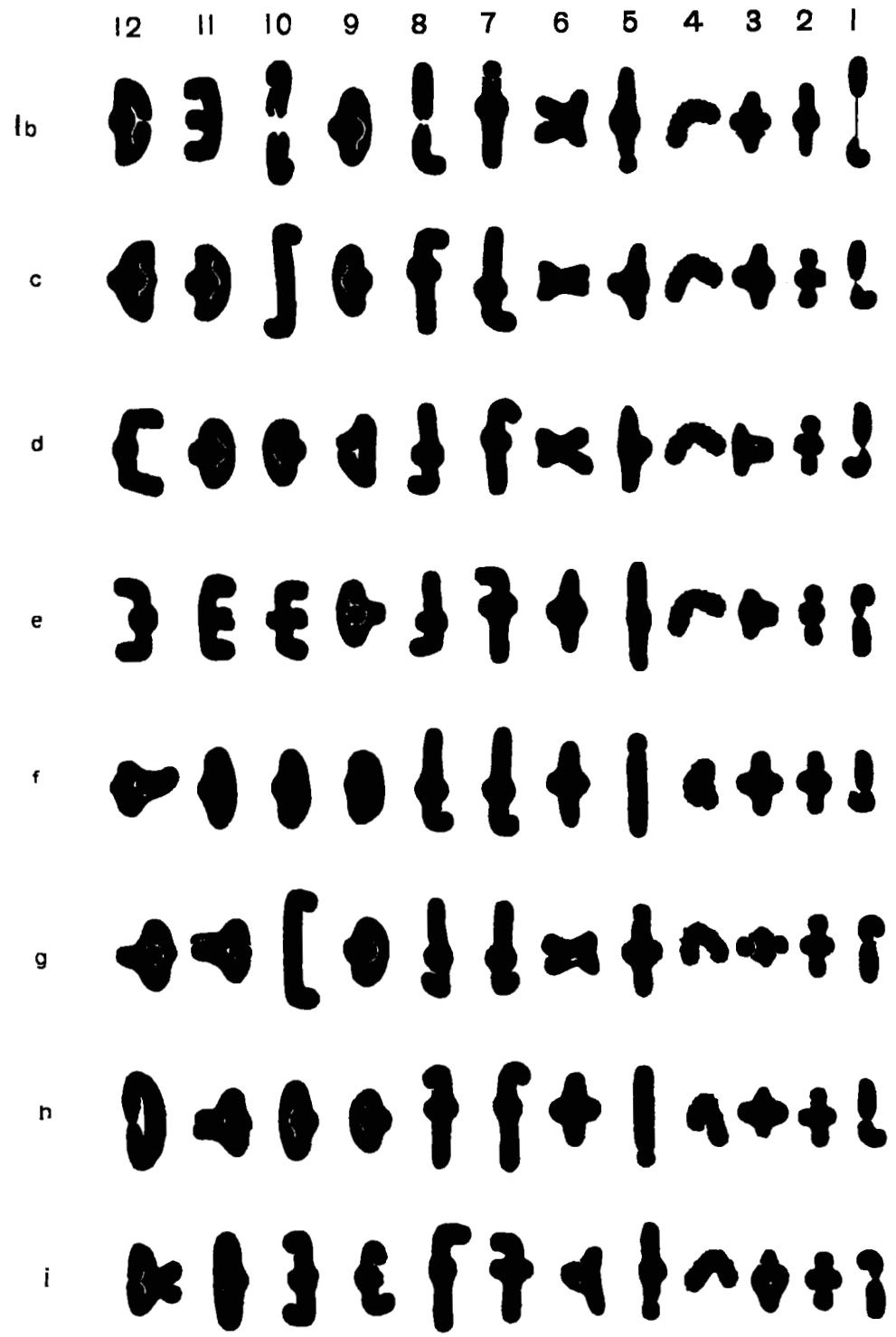
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PLATE 2

EXPLANATION OF FIGURES

Side view of first spermatocytes from eight individuals of form 'A,' showing variation of complex from individual to individual.

6 An animal with eight chromosomes of the Stenobothrus type.

7 A different individual with only four such chromosomes. The other four being replaced by J-shaped tetrads.

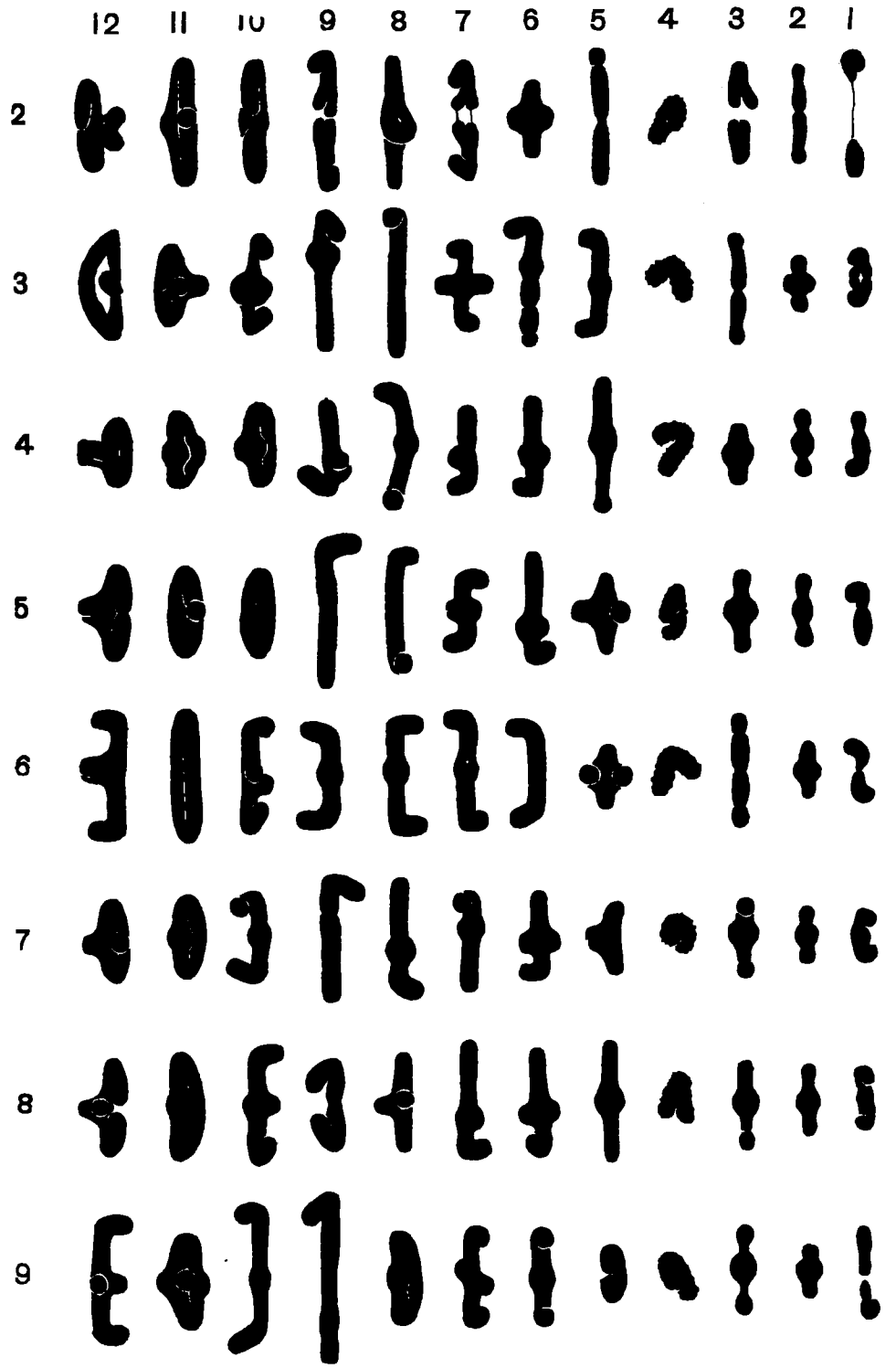
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PLATE 3

EXPLANATION OF FIGURES

Side views of eight first spermatocytes of form 'A' showing variation within the group.

10 Two supernumerary chromosomes, one large the other small.

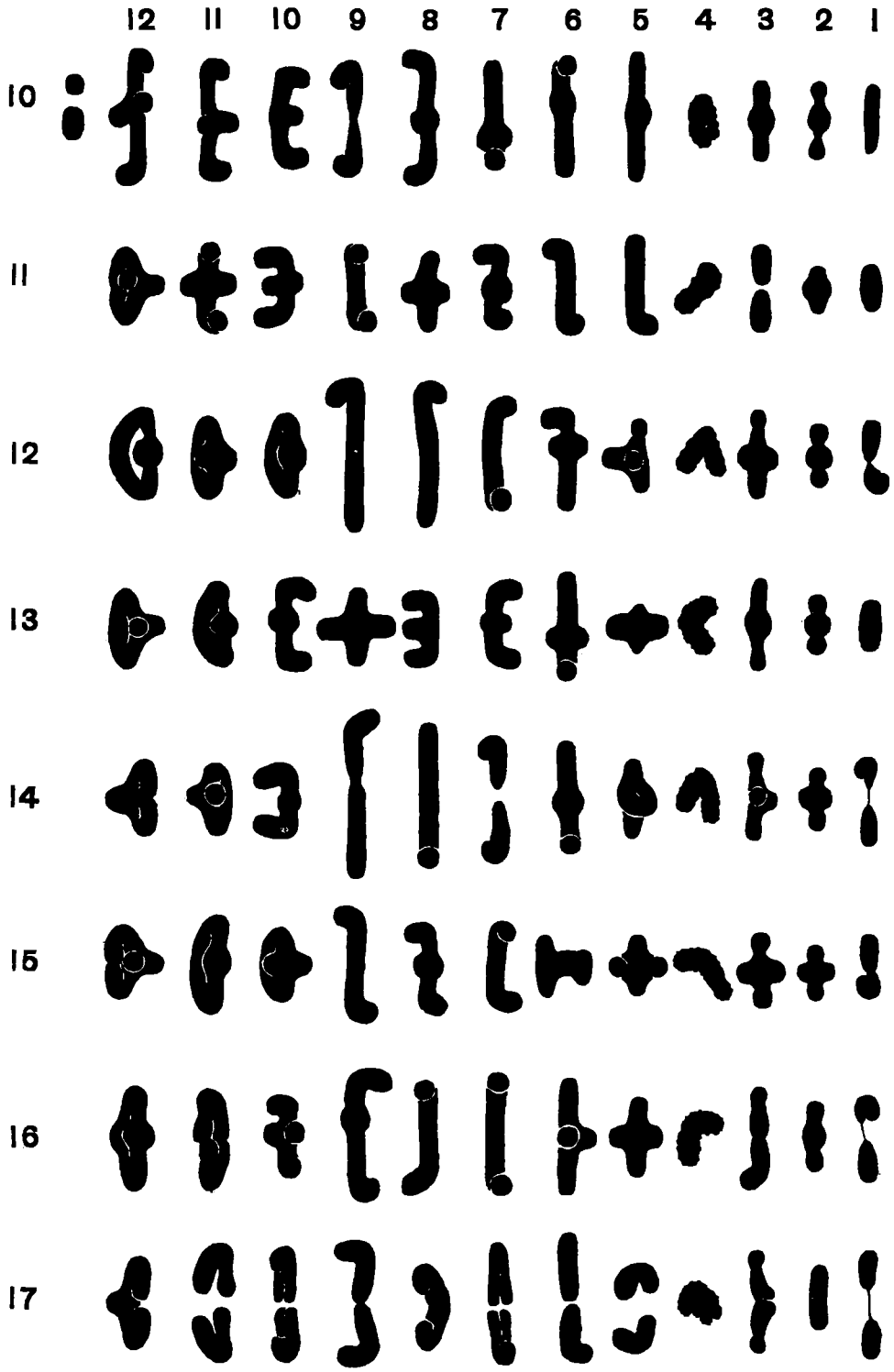
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PLATE 4

EXPLANATION OF FIGURES

Side views of first spermatocyte complexes from eight individuals of form 'A.' Showing variation within the group.

22 An animal showing hetermorphic condition of chromosomes number 11 and number 9.

23 Homomorphic short armed form of chromosome number 9.

24 Homomorphic long armed form of chromosome number 9 and heteromorphic form of chromosome number 11.

25 Homomorphic telomitic form of chromosome number 9.

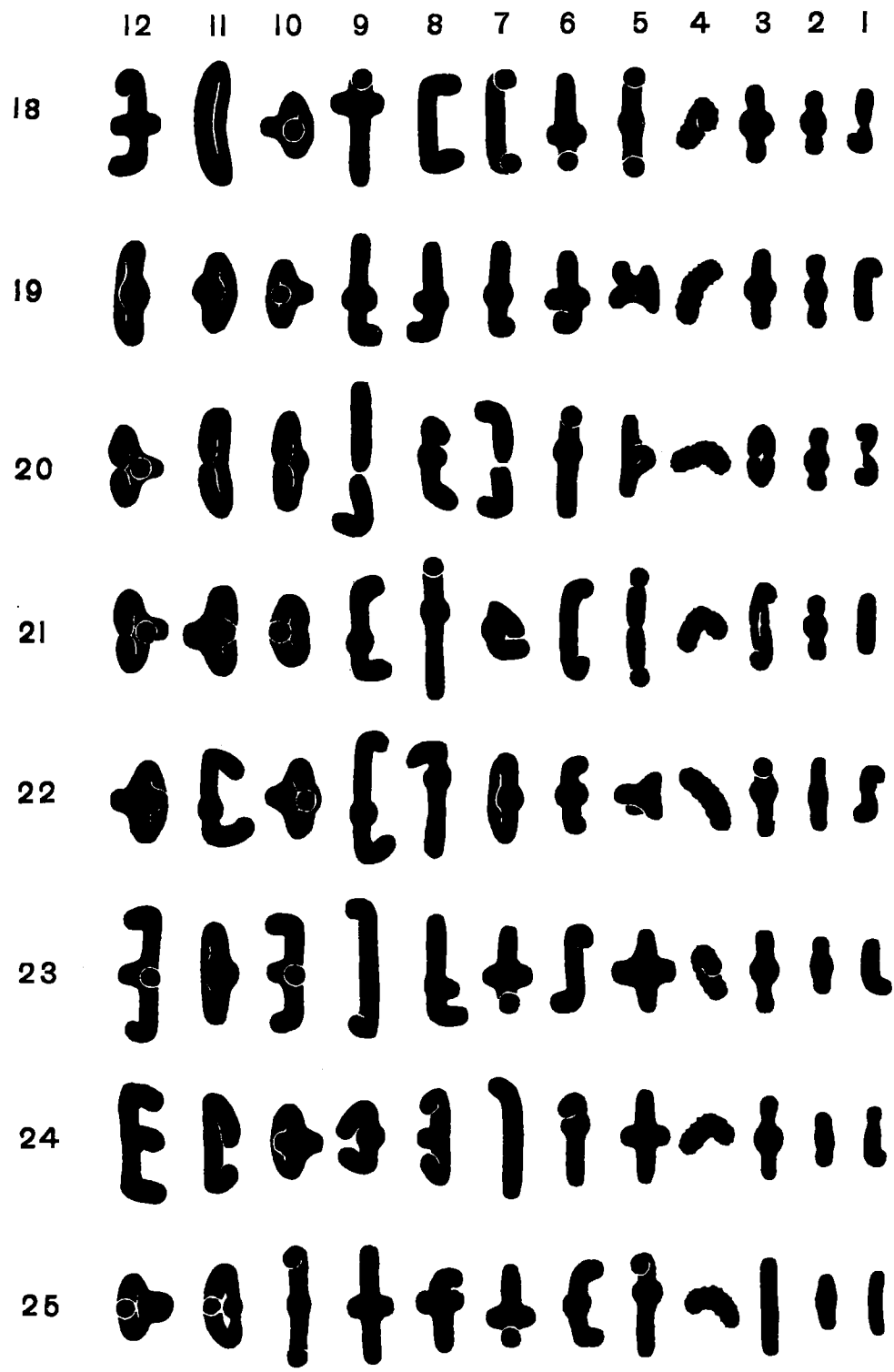
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PLATE 5

EXPLANATION OF FIGURES

Side views of seven first spermatocyte metaphases, form 'A,' showing variation within the group.

31 An individual with all of the chromosomes except numbers 1 and 2 either atelomitic or J-shaped.

32 An early anaphase with fifteen atelomitic dyads.

32*a*′ Spermatogonial metaphase with chromosomes arranged in pairs showing fifteen atelomitic chromosomes. From same animal as 32. Plate 11, figure 32*a*, is the same complex as drawn under the camera lucida.


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32a'

PLATE 6

EXPLANATION OF FIGURES

Side views of eight first spermatocyte metaphases, form 'B.' Note the difference in general appearance between plates 2 to 5 and 6 to 9, due to preponderance of atelomitic and J-shaped tetrads in former.

33 Chromosome number 4 (accessory) in two sections.

38 Chromosome number 10 broken.

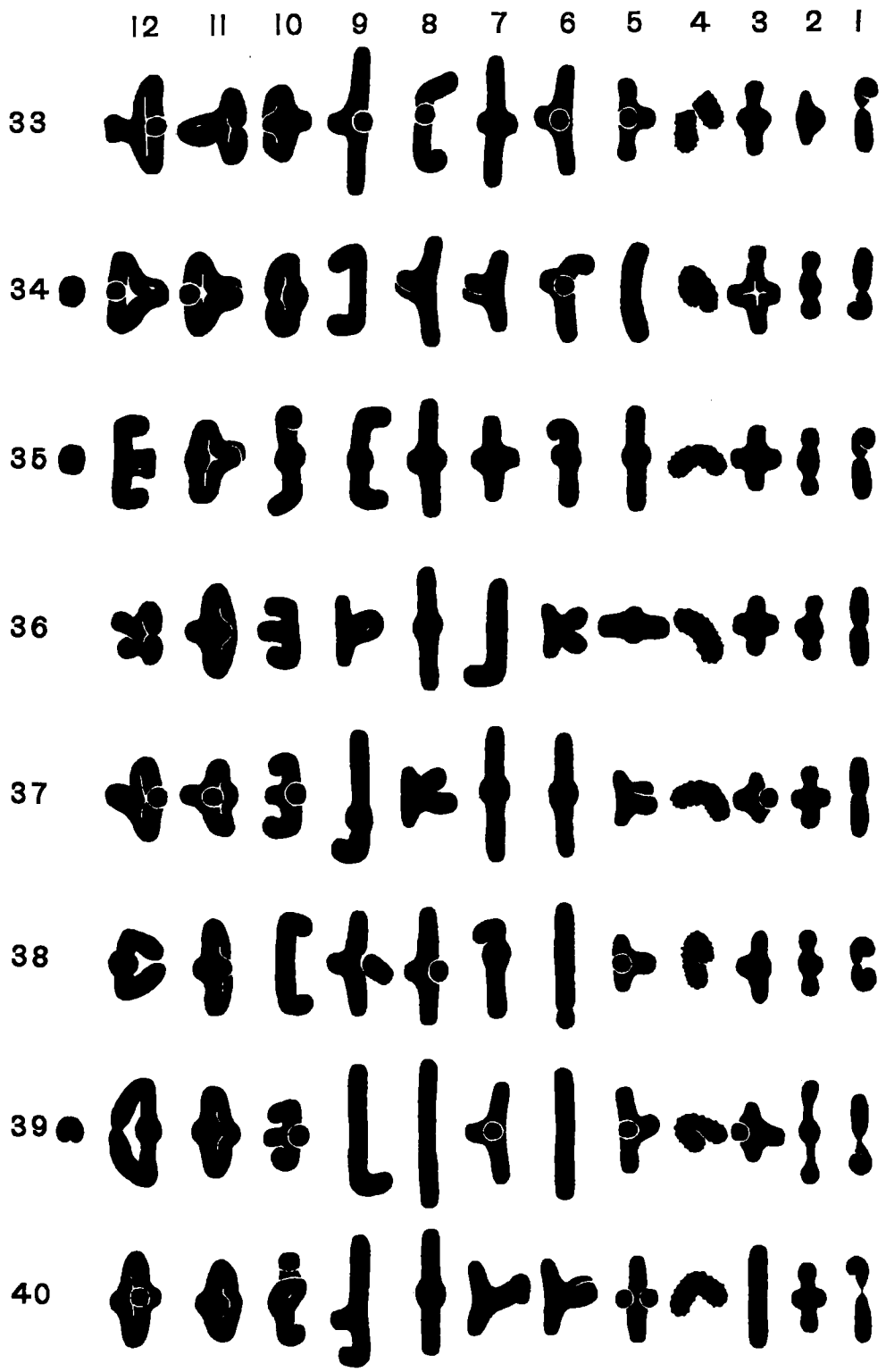
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PLATE 7

EXPLANATION OF FIGURES

Side views of eight first spermatocyte metaphases, form 'B.'

41 Chromosome number 4 (accessory) broken.

43 Two supernumeraries, one very large.

45 Accessory (no. 4) with numerous fiber attachments.

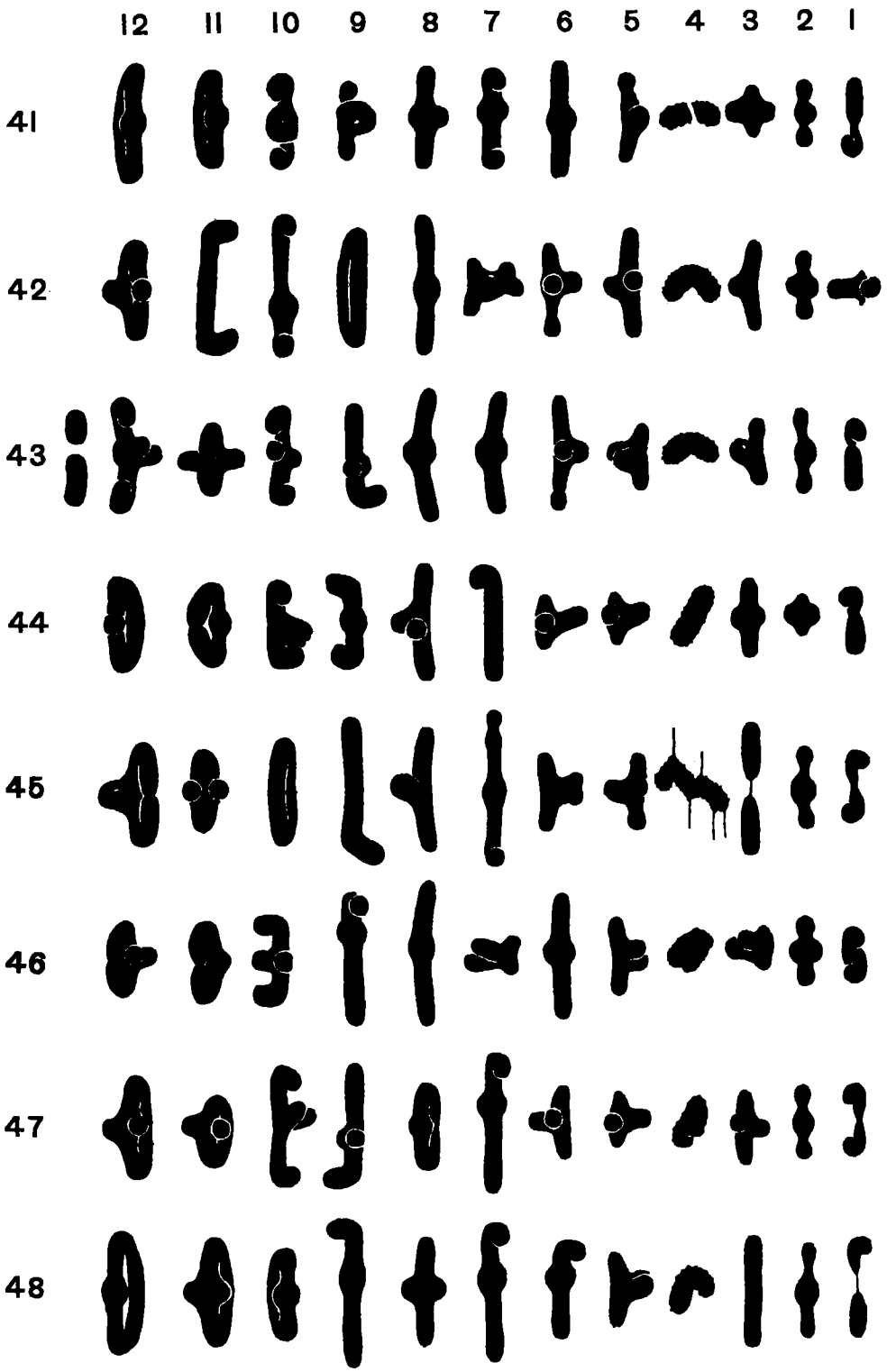
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PLATE 8

EXPLANATION OF FIGURES

Side views of eight first spermatocyte metaphases, form 'B.'
36 Two supernumeraries.

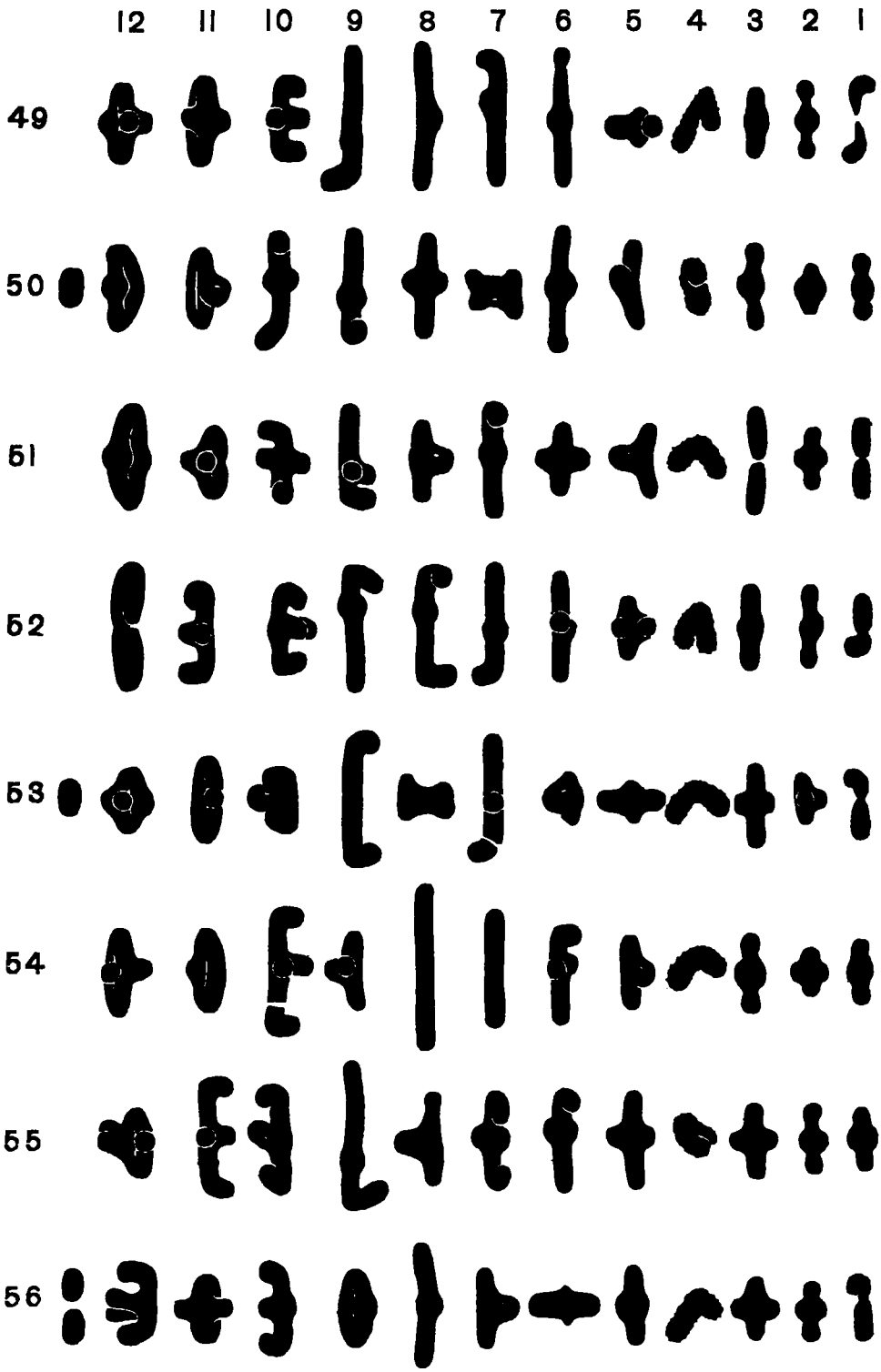
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PLATE 9

EXPLANATION OF FIGURES

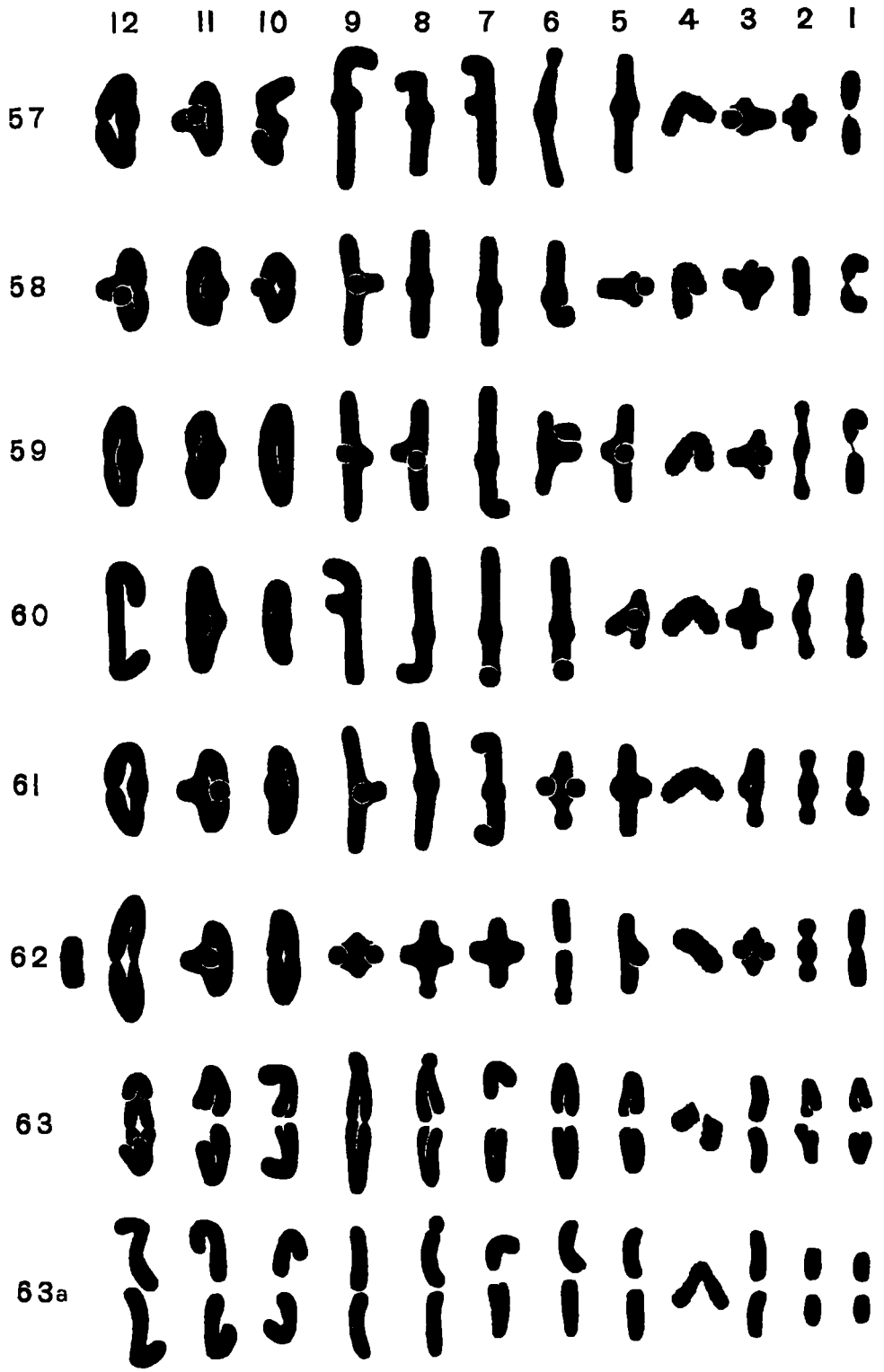
Side views of seven first spermatocyte metaphases, form 'B.'

62 This individual has the least number of atelomitic chromosomes found. Seven in the spermatogonia, resulting in three rings of the Stenobothrus type and the usual atelomitic accessory in the first spermatocyte. Note the very large supernumerary.

63 An early anaphase—chromosome number 4 cut.

63*a'* Spermatogonial metaphase with chromosomes arranged in pairs. From same animal as 63. Note the very close resemblance to the first spermatocyte anaphase especially, chromosome number 8, one homologue of which is constricted in each, and chromosome number 7, one homologue of which is atelomitic in both cases.

For the four classes of second spermatocytes formed by this individual, see plate 13, 63*b* to *e*.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63
63a

PLATE 10

EXPLANATION OF FIGURES

Side views of eight first spermatocyte metaphases of Circotettix lobatus. Note that there are only eleven chromosomes, number 2 being absent. None of these individuals have less than four rings of the Stenobothrus type.

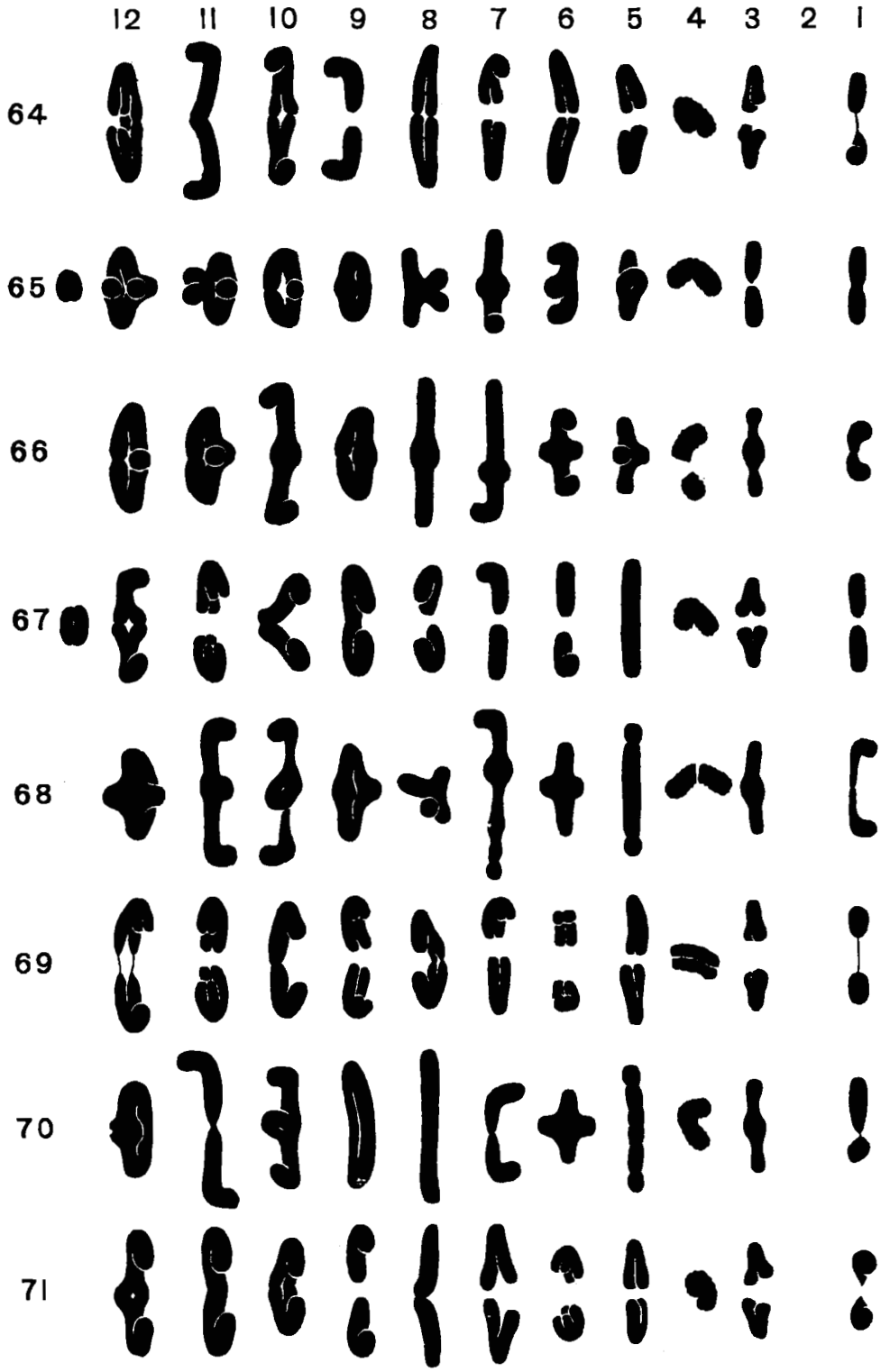
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PLATE 11

EXPLANATION OF FIGURES

Spermatogonial and somatic metaphases.

1*i* From the same animal as plate 1, twelve atelomitic chromosomes.

22*a* Seventeen atelomitic chromosomes. For first spermatocyte, see plate 4, 22.

32*a* Fifteen atelomitic dyads. For rearrangement and comparison with first spermatocyte, see plate 5, 32 and 32*a*′.

33*a* Ten atelomitic chromosomes.

38*a* A tetrad dividing unequally.

62*a* Seven atelomitic chromosomes, the minimum number.

62*b* Another complex from above individual.

63*a* Eight atelomitic chromosomes. For rearrangement and comparison with first spermatocyte, see plate 9, 63 and 63*a*′.

70*a* Circotettix lobatus. Entire complex, twenty-one chromosomes.

70*d* Prophase tetrad showing vesicles on one homologue.

70*e* Unequal division of same tetrad in first spermatocyte metaphase.

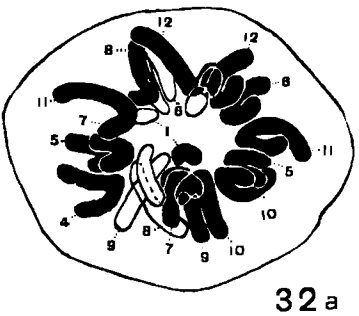
75 Circotettix lobatus. Entire complex, twenty-one chromosomes.

72 Somatic complex from a female of form 'B,' ten atelomitic chromosomes.

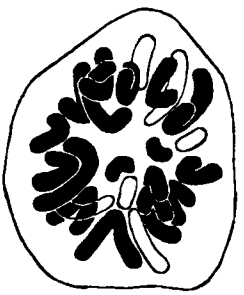
73 From another female of same group, eight atelomitic chromosomes.

74 From a female of form 'A,' fourteen atelomitic chromosomes.

1i

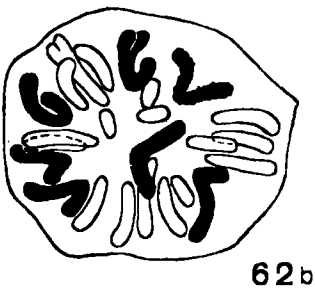


32a

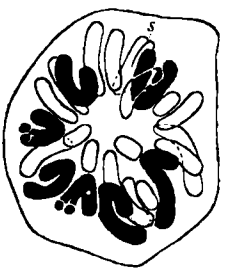


22a

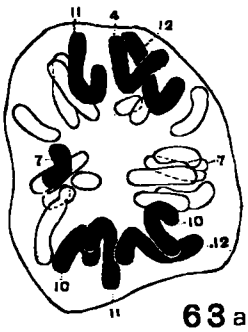
33a



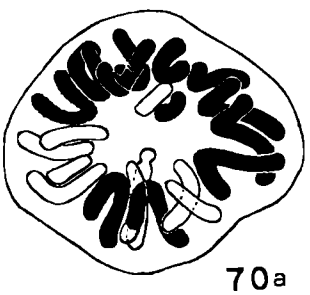
62b



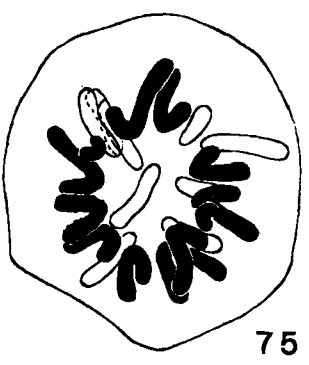
62a



63a

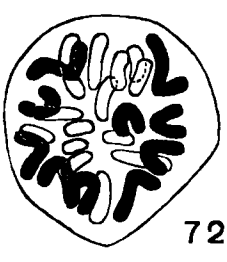


70a



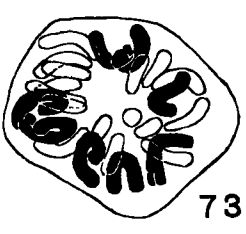
75

38a 70d 70e



72

74



73

PLATE 12

EXPLANATION OF FIGURES

Second spermatocyte metaphases from animal number 1 showing twelve morphologically different types, as follows:

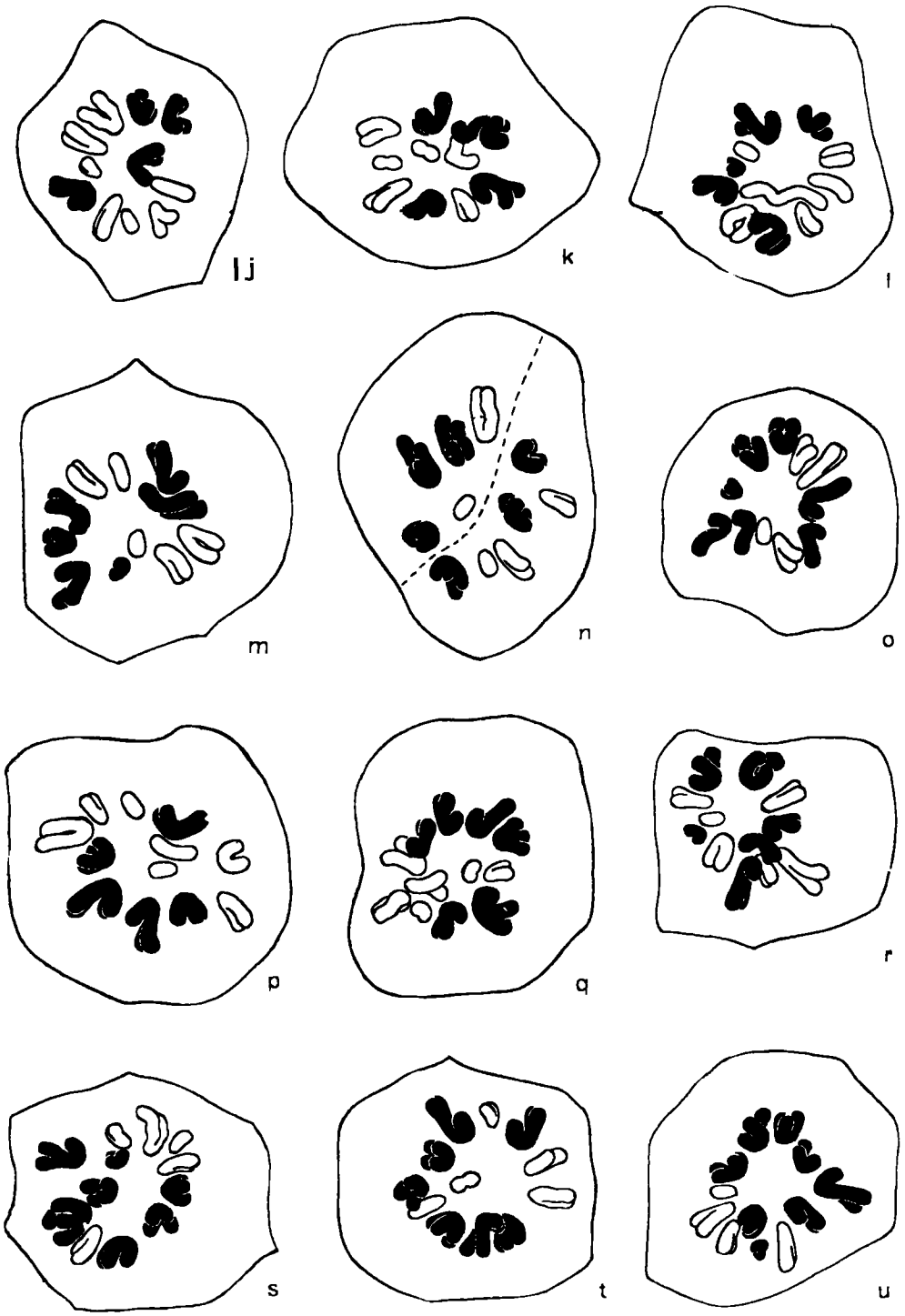
Eleven chromosomes

1. *j*, four V's plus seven rods.
   k, four V's plus six rods plus V of tetrad number 7 or 8.
   l, four V's plus six rods plus V of tetrad number 1.
   m, four V's plus five rods plus V's of numbers 1 and 7 or 8.
   n, four V's plus five rods plus V's of numbers 7 and 8.
   o, four V's plus four rods plus V's of numbers 7 and 8.

Twelve chromosomes

   p, five V's plus seven rods.
   q, five V's plus six rods plus V of tetrad number 7 or 8.
   r, five V's plus six rods plus V of tetrad number 1.
   s, five V's plus five rods plus V's of numbers 1 and 7 or 8.
   t, five V's plus five rods plus V's of numbers 7 and 8.
   u, five V's plus four rods plus V's of numbers 1, 7 and 8.

For first spermatocyte metaphases from this animal, see plate 1 and for spermatogonia plate 11, 1 *i*.


j
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l
m
n
o
p
q
r
s
t
u

PLATE 13

EXPLANATION OF FIGURES

Second spermatocyte metaphases.

62*b* to *c* From the individual with the minimum number of atelomitic chromosomes, seven in spermatogonia (Plate 11, 62*a*). For first spermatocyte see Plate 9, 62. There are four types owing to the presence of the supernumerary as follows:

b, eleven chromosomes.

c, eleven chromosomes plus supernume ary.

d, twe ve chromosomes.

e, twelve chromosomes plus supernumerary.

63*b* to *e* The four classes of second spermatocytes formed by individual 63.

69 Circotettix lobatus, eleven chromosomes.

69*b* Same, ten chromosomes.

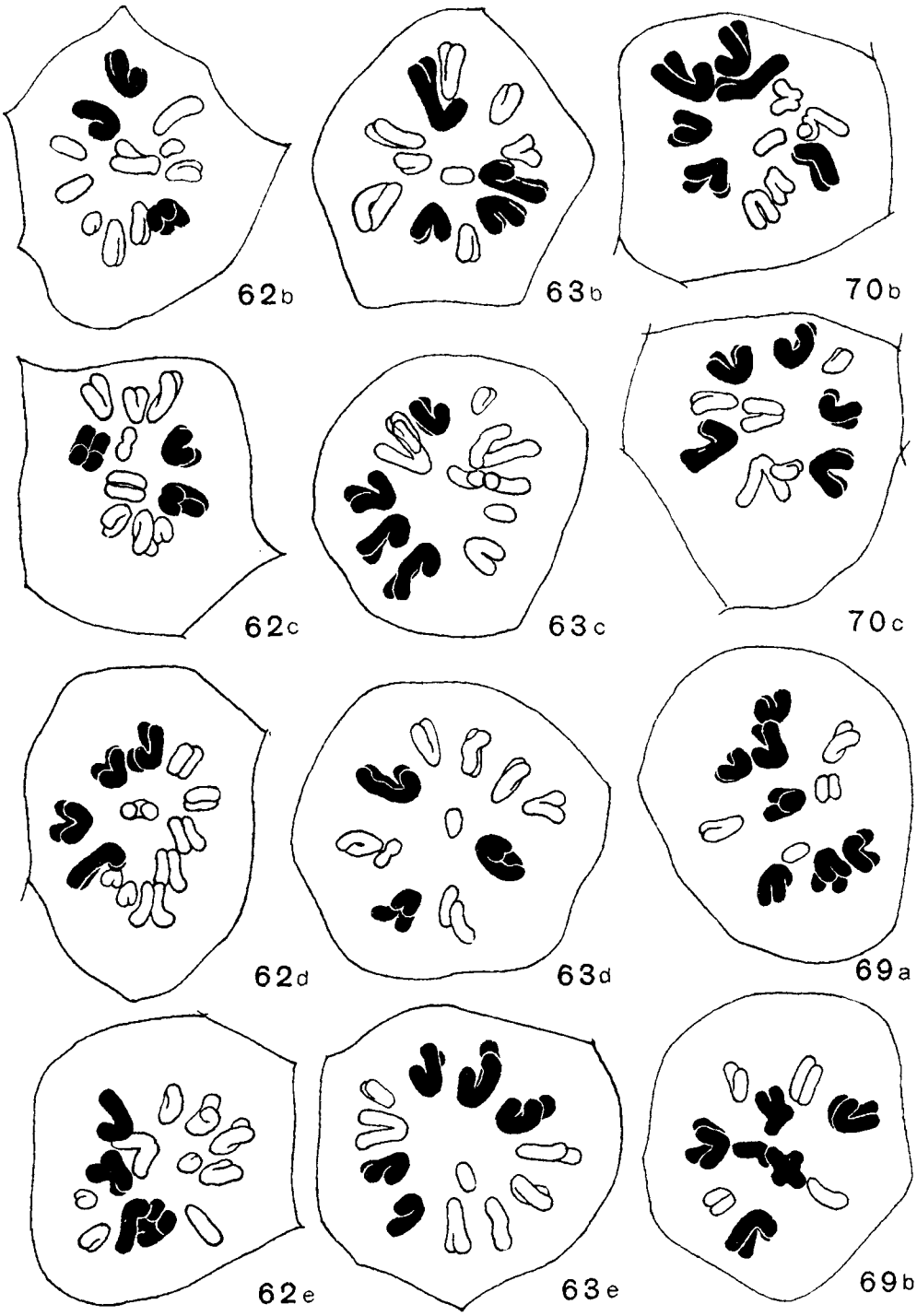

62b
63b
70b
62c
63c
70c
62d
63d
69a
62e
63e
69b

PLATE 14

EXPLANATION OF FIGURES

Photomicrographs

21*a* Homomorphic constricted type of chromosome number 3.

62*f* Heteromorphic form of same chromosome from another individual—one homologue constricted the other smooth.

13*a* Homomorphic smooth type of same chromosome from a third animal.

1*v* The concurrent passage of the atelomitic homologues of chromosomes number 7 and 8 to the opposite pole from the accessory in specimen number 1.

1*w* The same except that the atelomitic dyads are passing to the same pole as the accessory.

1*x* The three J-shaped tetrads of animal number one with alternate distribution of atelomitic dyads of chromosomes number 7 and 8.

22*b* Homomorphic, atelomitic form of chromosome number 1.

22*c* Heteromorphic condition of chromosome number 11.

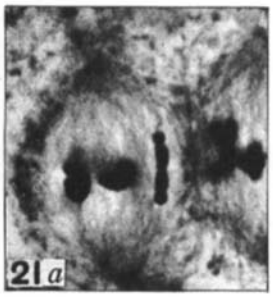
23*a* Homomorphic long-armed form of chromosome number 11.

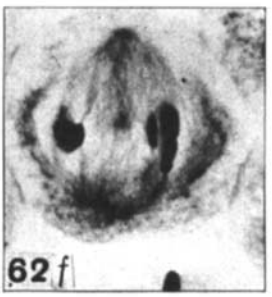
10*a* Homomorphic short-armed form of chromosome number 11.

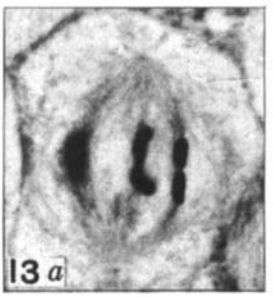
22*d* Heteromorphic condition of chromosome number 9.

17*a* Homomorphic long-armed form of chromosome number 9.

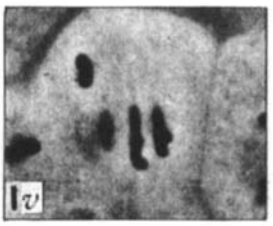
10*b* Homomorphic short-armed form of chromosome number 9.


21a

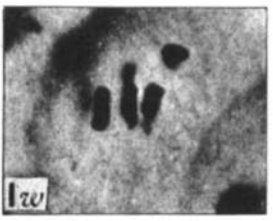

62f


13a


1x


1v


22b


1w


23a


22c


10a


17a


22d


10b